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FACTORS AFFECTING SUSCEPTIBILITY TO DISEASE IN PLANTS.

PART I.

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THE problem of immunity to disease in plants is now receiving a large amount of attention from plant-pathologists. It has long been known that different varieties of the same species of plant may differ in their susceptibility to the attacks of fungi and possibly other parasites, but even now practically nothing is known of the factors which determine whether a plant will prove immune to a disease or not. The following paper is a record of investigations which it was hoped would contribute something towards the solution of this problem, or which might at least suggest lines of further research on this subject.

It was thought that the simplest method of starting an attack on this question was by finding what effect the nutrition of a plant had on its susceptibility to disease. There has long been a general impression that heavy manuring makes plants more liable to disease, but hitherto no definite data have been available nor any detailed experiments made.

From experiments on the effect of mineral starvation on Bromes Marshall Ward[1] concluded that decreased susceptibility to rust was only caused by the lack of sufficient food in the host plant on which the rust fungus could feed, i.e. that the host was just as susceptible as under normal conditions but could not supply food to so large a quantity of the rust fungus. Later, histological examinations also showed that fungal hyphae in an immune plant were adversely affected in the same way as in a starved susceptible plant[2].

[1] M. Ward, *Proc. Roy. Soc.* 71, 1903. [2] M. Ward, *Ann. Bot.* 19, 1905.

These seemed to be the only records of any investigations on the effect of nutrition on immunity to disease in the higher plants. It was therefore decided to grow numbers of wheat plants under varying conditions of nutrition and to observe any differences in their susceptibility to the attacks of disease.

The first set of experiments was performed with a number of wheat plants grown in water-cultures. The wheat grains were germinated on damp blotting-paper and were transferred, when the plumules were about an inch long, to the water-cultures. The young plants were supported in split corks in the necks of the bottles which had a capacity of about 300 c.c.

Throughout the experiments the bottles were filled up with tap-water on alternate days to replace the water lost by transpiration: the solution was left in the bottles for ten days without any attention except the filling-up above mentioned, and was then poured out and replaced by clean tap-water. After two days the bottles were filled with fresh nutrient solutions. These operations were repeated throughout the experiment, so that the plants received fresh solutions every twelve days but were in tap-water only for the last two days of each twelve-day period. It should be mentioned that the plants grew exceedingly well under these conditions and were indeed remarkable for their strong growth and extensive tillering. All the bottles received the same standard nutrient solution but to different series were added various small amounts of strong solutions of different salts. The standard solution employed was the one used by Detmer and made up as follows:

Calcium nitrate	1 gm.
Potassium chloride	·25 gm.
Magnesium sulphate	·25 gm.
Potassium dihydrogen phosphate	·25 gm.
Ferric chloride	a few drops of solution.
Tap-water	1000 c.c.

Fifteen series of cultures were grown, each series consisting of three plants of a variety of wheat highly susceptible to *Puccinia glumarum* (Michigan Bronze) and three of a variety almost immune to the attacks of this fungus (Little Joss).

Series 1 was supplied with the normal nutrient solution, the 300 c.c. of solution which was placed in each bottle containing ·051 gm. nitrogen in the form of nitrate, ·017 gm. phosphorus as phosphate, and ·06 gm.

potassium. In series 2 the amount of nitrogen was doubled by adding ·3 gm. sodium nitrate, containing ·051 gm. nitrogen, to the solution in each bottle, while in series 3 the amount of nitrogen was increased to four times the normal by adding ·9 gm. sodium nitrate. The next two series, 4 and 5, also contained double and four times the normal amount of nitrogen, but in this case the additional nitrogen was contained in ·24 gm. and ·72 gm. of ammonium sulphate respectively. To series 6 and 7 was added sodium phosphate, the addition of ·077 gm. containing ·017 gm. phosphorus doubling the phosphorus in series 6, and of ·23 gm. making the phosphate four times the normal in 7. Similarly the potassium in series 8 and 9 was increased to twice and four times the normal by adding ·115 gm. and ·345 gm. of potassium chloride, that is ·06 and ·18 gm. potassium, to series 8 and 9 respectively. Series 10 and 11 received only the normal solution but the concentration in 10 was double, and in 11 four times the normal concentration. On the other hand series 12 and 13 received the normal solution diluted to one half and one quarter the original concentration. Series 14 and 15 received an addition of both potassium and phosphate; in the first case the potash and phosphate were both brought to twice the normal, and in the second case to four times.

The seedlings were placed in the water-cultures on Feb. 28 in the case of the series 1—9, and a week later in the case of the remaining series, the whole set of cultures being grown close together in the same greenhouse.

As may be deduced from the varieties of wheat selected, it was originally intended to inoculate the plants with *Puccinia glumarum*, but as late as April 24 it had been found impossible to obtain enough to infect the plants. Only a very small quantity of this fungus was obtained on April 24 and a few inoculations were made, but these proved to be failures. In the meantime a spontaneous outbreak of *Erysiphe graminis* had occurred among the plants, so it was resolved to study the attack of this parasite instead of that of *Puccinia glumarum*. Accordingly the spread of the mildew was encouraged by putting the plants close together and keeping the atmosphere in the greenhouse damp. Also, the relative positions of the plants were frequently changed so as to give the healthy every opportunity of being attacked, and at the same time to eliminate any differences due to the outside plants receiving more air and light.

On April 24 all the plants were examined and marks were awarded to each in proportion to the amount of mildew present, those with only

a trace of mildew being marked 1 and those which were most severely attacked being marked 10. This examination and marking was repeated on May 3 and for the third and last time on May 15. By this time the most badly attacked plants were covered all over with a thick coating of mildew and were practically dead: their condition can be seen from the photograph in Plate VII, Fig. 1. The least diseased plants only showed a few patches of mildew and their leaves were strong and of a good green colour, contrasting very strongly with the dingy flaccid leaves of the badly diseased plants. Fig. 2, Plate VII, shows one of the healthiest plants.

It must be remembered that these comparatively healthy plants had been surrounded by, and actually in contact with, the badly diseased ones for several weeks and had thus had every opportunity of succumbing to the attack of the parasite. Most of the plants grew to about the same size, the exceptions being in the series 5, 11, 12 and 13.

The plants in series 5, which received a large addition of ammonium sulphate, were of the average size as regards their leaves but their root-systems were distinctly poor. The plants growing in the concentrated solution in series 11 were rather smaller and their roots were rather below the average size. The plants which grew in solutions more dilute than the normal (12 and 13) were small but had well-developed roots.

The intensity of disease increased on all the plants between the first and last occasion of marking, but as the relative positions of the plants in the scheme of marking were practically constant only the final markings are given here.

Table I shows the marks which indicate the amount of disease on each of the 90 plants at the conclusion of the experiment.

It will be seen from the preceding table that the nutrient solutions in which the plants were growing exercised a very large influence on their susceptibility to disease, and that the different degrees in which the plants became diseased was not accidental but can be correlated with the treatment they received. In the first place it will be noted that although one variety of wheat was more susceptible to disease than the other, yet the effect due to the different solutions follows almost the same relative order in both varieties. In both cases series 2, 3, 4 and 5 which received additional nitrogen contained the most badly diseased plants (Plate VII, Fig. 3), though it is curious that the plants of No. 5 receiving four times the normal amount of nitrogen as ammonium sulphate were not so badly attacked as those of No. 4 which only

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

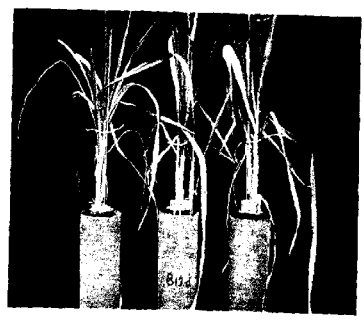

Fig. 6.

received twice the normal amount of nitrogen: perhaps there is some connection between this fact and the stunted root-growth noticed in series 5.

TABLE I.

No.	Solution	Degree of disease					
		Little Joss (3 plants)			Michigan Bronze (3 plants)		
1	Normal	9	7	7	10	5	5
2	Nitrogen × 2 $NaNO_3$	9	9	9	10	7	7
3	,, × 4 $NaNO_3$	10	10	8	10	7	5
4	,, × 2 $(NH_4)_2SO_4$	10	10	10	9	9	9
5	,, × 4 $(NH_4)_2SO_4$	8	8	8	8	8	5
6	Phosphate × 2	6	6	4	6	5	5
7	,, × 4	7	7	7	5	5	2
8	Potash × 2	7	5	4	5	5	2
9	,, × 4	5	5	3	3	3	3
10	Normal × 2	9	9	9	9	7	7
11	,, × 4	9	8	8	5	5	5
12	,, × ½	7	7	5	1	1	1
13	,, × ¼	7	5	5	1	1	1
14	Phosphate and potash × 2	7	7	7	5	3	3
15	,, ,, ,, × 4	7	4	4	7	5	5

Next, the plants receiving a concentrated nutrient solution were observed to be badly diseased, but here again the extremely strong solution in series 11 has checked the growth of the plants to some extent, and at the same time there is less disease than in the plants of No. 10 (Plate VII, Fig. 4).

Series 6 and 7 receiving additional phosphate showed about the average amount of disease, or possibly rather less. The series 8 and 9 which received additional potash were very clearly rendered less susceptible to disease: in the case of Little Joss the plants of Nos. 8 and 9 were the healthiest of the whole set (Plate VII, Fig. 5), while among the Michigan Bronzes they were the healthiest with the exception of those of series 12 and 13.

The plants receiving additional phosphate and potash (14 and 15) seemed to be intermediate in their susceptibility between those receiving only phosphate or only potash.

The plants growing in dilute solutions (12 and 13) were healthier than the normal, and indeed in the case of the Michigan Bronzes they were the healthiest of the set (Plate VII, Fig. 6): in both varieties the plants of series 13 were slightly less diseased than those of 12; that is, the disease was reduced when the food supply was reduced.

While the above-mentioned water-cultures were in progress a parallel series of experiments was being made on wheats grown in soil.

A number of wooden boxes about 17 inches × 13 inches were filled to a depth of $2\frac{1}{2}$ inches with rather poor soil which was obtained by mixing a rather rich soil, obtained from the neighbourhood of the laboratory, with about an equal bulk of sand. In one box the soil was made still poorer by doubling the amount of sand.

On March 12 twelve grains of Little Joss were sown in each box and twelve of Michigan Bronze, two rows of each variety in each box: on the average eleven grains of each variety produced plants. The boxes were all in the same greenhouse near the water-cultures, so that the plants were very readily naturally infected by the mildew, and in this case also the position of the boxes was often changed in order to make the conditions as far as possible equal for all.

An attempt was made to add artificial manures to the boxes in amounts comparable to those used in practice in the field. It was calculated that the area of a box was $1\frac{1}{2}$ square feet or $\frac{1}{30,000}$ acre and that 1·7 grams per box would be equivalent to 1 cwt. per acre. The quantities of fertilisers applied are given in Table II which also records the results. The salts to be added were made into solutions and it was intended to apply these solutions in six doses at intervals of a fortnight, but the experiment was brought to an end when only four of these doses had been applied. This explains the fractions in the amounts of fertiliser applied; thus 1·33 cwt. was originally intended to have been increased to 2 cwt., and so on.

The plants in all the boxes grew almost equally well, but before the mildew had attacked them seriously those which received nitrogenous manures were larger and of a darker colour than the rest: on the other hand the plants in the "starvation" box were small, thin and light-coloured. As in the case of the water-cultures the boxes were examined and graded according to the amount of mildew present on May 4, 15 and 20, but as the relative amounts were constant only the last set of marks is given here. The plants in each box were not marked individually, as the individuals of the same variety in the same box showed no differences, but the two varieties in each box were judged separately.

The extent of the disease on the plants in each box is shown in Table II.

It will be seen that the order of the severity of the attack of mildew in the different boxes was the same for both varieties of wheat.

The plants in box 5 receiving a double dressing of sodium nitrate were the most severely attacked in both cases, while all the other boxes which received nitrogenous manures showed a very bad attack. The least susceptible plants were those in box 9, which received a double dose of sodium phosphate, while those in 10 and 11 which received potassium chloride were only slightly attacked, the same being the case in box 14 where the plants were almost starved.

TABLE II.

No.	Treatment	Degree of disease	
		Little Joss	Michigan Bronze
1	No manure	7	5
2	Complete manure 2·3 gm. $NaNO_3$ equivalent to 1·3 cwt. $NaNO_3$ per acre 3·4 ,, Na_2HPO_4 ,, ,, 3·3 ,, "super" ,, ·27 ,, KCl ,, ,, 1·3 ,, kainit ,,	9	7
3	Complete manure. Double the above quantities	9	7
4	2·3 gm. $NaNO_3$ equivalent to 1·3 cwt. $NaNO_3$ per acre	8	6
5	4·6 gm. $NaNO_3$ equivalent to 2·6 cwt. $NaNO_3$ per acre	10	8
6	1·8 gm. $(NH_4)_2SO_4$ eqnivalent to 1 cwt. $(NH_4)_2SO_4$ per acre	8	6
7	3·6 gm. $(NH_4)_2SO_4$ equivalent to 2 cwt. $(NH_4)_2SO_4$ per acre	9	7
8	3·4 gm. Na_2HPO_4 equivalent to 3·3 cwt. "super" per acre	6	4
9	6·8 gm. Na_2HPO_4 equivalent to 6·6 cwt. "super" per acre	3	1
10	·27 gm. KCl equivalent to 1·3 cwt. kainit per acre	4	2
11	·54 gm. KCl equivalent to 2·6 cwt. kainit per acre	5	3
12	3·4 gm. Na_2HPO_4 equivalent to 3·3 cwt. "super" per acre ·27 ,, KCl ,, ,, 1·3 ,, kainit ,,	7	5
13	Double the quantities in No. 12	6	4
14	No manure. Double the quantity of sand	5	3

A single dressing of sodium phosphate seems to be less useful in preventing disease than a single dressing of potassium chloride, but if the dose be doubled the result is reversed.

A mixture of sodium phosphate and potassium chloride also seems to be rather less effective than either of these substances separately.

On the whole these results agree very closely with those obtained from the water-cultures, though phosphates appear to be more effective in checking disease when the plants are grown in soil than when grown in water-cultures.

Two more sets of wheat-cultures were carried out in a similar manner to the preceding ones in order to find the effect of nutrition on the susceptibility of the plants to yellow rust, *Puccinia glumarum*.

The first of these was a set of water-cultures in which the same varieties of wheat as before were used, but a much smaller number were employed. In this case only those culture solutions were used which had been found to give extreme results in the first investigation, together with one of the so-called normal solutions as a control. There were then three series of cultures, each consisting of three plants of each variety, and they were treated in identically the same way as the corresponding cultures of the former set. The only difference was that this set of cultures was carried out in the open air as far from the greenhouse as possible in order to avoid infection by the mildew: but it was found impossible to keep them free from the mildew, though they were not nearly so badly attacked as those on which this parasite had been encouraged.

The plants of series 1 received the normal solution (cf. series 1 of former set); those of series 2 had the amount of nitrogen in the solution doubled by the addition of ammonium sulphate (cf. series 4 above), and those of series 3 were grown in a solution containing four times the normal amount of potash (cf. series 9).

On May 23 the seedling wheats were placed in the water-cultures and on May 28 when each showed two leaves they were inoculated with uredospores of *Puccinia glumarum*. The inoculations were made by scraping a "rusty" leaf of wheat with a wet penknife-blade and then applying the blade to the tips of the leaves to be infected; the leaf-tips were first made moist by blowing on to them through a narrow glass tube which caused the water containing the spores to adhere more readily to the leaf. On June 6 more inoculations were made, but this time on the middle of the upper surface of the leaf-blades: there were at this time three or four leaves on each plant.

On June 10 rust pustules appeared on one plant on an inoculated spot, and on June 12 eight plants showed pustules. From this date the disease spread rapidly without any artificial assistance other than that of placing the plants very close together so that the rusty leaves could touch the healthy ones.

The plants were graded for rust on July 1 and it is interesting to note that at this time all the plants showed rust pustules, though the Little Joss, which is almost immune, had the disease to a very much less extent than the Michigan Bronze. On July 12 the plants were again marked for the rust attack and the marks are shown in Table III.

TABLE III.

No.	Solution	Degree of rust					
		Little Joss			Michigan Bronze		
1	Normal	0	0	0	8	6	5
2	Nitrogen × 2 (Amm. sulph.)	0	3	3	9	9	8
3	Potash × 4	0	0	2	6	5	3

It will be seen that only three out of the nine plants of the relatively immune variety showed any rust at this stage, and of these two were those which had received solutions with double nitrogen. With one exception all the Michigan Bronze plants were much more diseased than any of the other variety, and it will be seen that the attack on series 2 was much worse than that on 1, while on series 3 it was slightly less. By this time all the plants of the first variety were suffering severely from mildew, so they were thrown away: but the plants of the other variety were kept for a few weeks longer. On July 23 the remaining plants were again marked and found to occupy the same relative positions as in the previous marking. On Aug. 5 however the rust on the plants of series 1 and 2 was the same as on the previous occasion, while two of the plants in series 3 were much more severely attacked than before, earning marks 8 and 6. Perhaps this can be accounted for by the fact that the plants of 1 and 2 were almost killed by mildew and so the rust had no chance to spread further, while plants of series 3 were still green and growing vigorously.

Observations were also made on the susceptibility to rust of a number of wheat plants grown in pots receiving different nutritive solutions. Fourteen series of 5-inch pots were grown, each series consisting of one pot containing three plants of Little Joss and one containing three plants of Michigan Bronze. The amounts of manurial substances to be added to the various pots were calculated on the same

system as was employed in the case of the boxes. As before, a soil mixed with about an equal amount of sand was used in all the pots.

The fourteen series of pots were treated in the following ways: No. 1 was a control receiving no manure; Nos. 2, 3 and 4 received different quantities of a complete manure; Nos. 5 and 6 received respectively a single and double dose of sodium nitrate, while 7 and 8 were given the same amounts of nitrogen in the form of ammonium sulphate. To Nos. 9 and 10 were given single and double dressings of sodium phosphate, and Nos. 11 and 12 received similar treatment with potassium chloride. Nos. 13 and 14 received no manure, but were sterilised in a steamer at 100° C. for two hours before the seeds were set. The manures used were equivalent to about 2 and 4 cwt. sodium nitrate per acre, 1½ and 3 cwt. ammonium sulphate, 5 and 10 cwt. superphosphate, and 2 and 4 cwt. kainit. The fertilisers in solution were added in three separate doses, an interval of about a fortnight occurring between the applications, and the first dressing was given about a week after sowing the seeds.

The seeds were sown in the pots on May 8 and by May 20 all the seeds had germinated and each seedling showed one or two leaves. Between May 18 and 23 every leaf on all the plants was inoculated with uredospores of *Puccinia glumarum* in the manner previously described. The first sign of successful infection was seen on May 28, when one leaf was found to bear some unbroken pustules on its tip: these pustules opened and set free their spores on the following day. More leaves gradually showed pustules until on June 5 the disease was evident on Little Joss plants in four pots, and on Michigan Bronze plants in eight pots. On June 6 another inoculation was made, this time on the middle of the upper surface of the leaves, about four leaves being inoculated in each pot.

By June 10 all the Michigan Bronze plants showed rust, while the disease had appeared on about half the Little Joss plants. From this date onward the disease spread rapidly and on July 1 the cultures were first graded according to the amount of rust present on each. At this time ten of the fourteen Little Joss cultures were marked 0 as being free from rust, while the remaining four were marked 1 or 2, the disease attack being very slight. Among the Michigan Bronze cultures 9, 10, 11 and 12 were the least diseased, all the others being almost equally bad, although perhaps 3, 7 and 8 were the worst.

On July 10 the marks awarded were almost the same as before, but

although there were again four plants of Little Joss showing degrees 1 or 2 of rust three of these were not included in the four on July 1.

The plants were again marked according to their rustiness on July 23, Aug. 5 and 13, but the disease was making no further progress and the relative amounts of rust on the various plants showed only small fluctuations which might almost be put down to errors in observation. The figures in Table IV may therefore be considered to represent the extent of the disease on the different cultures when the disease had attained its maximum development.

TABLE IV.

No.	Manure equivalent per acre	Extent of disease	
		Little Joss	Michigan Bronze
1	None	0	6
2	1 cwt. sodium nitrate 2½ ,, "super" 1 ,, kainit ...	1	7
3	2 ,, sodium nitrate 5 ,, "super" 2 ,, kainit ...	1	7
4	4 ,, sodium nitrate 10 ,, "super" 4 ,, kainit ...	1	7
5	2 ,, sodium nitrate	1	7
6	4 ,, ,, ,, ...	1	7
7	1½ ,, ammonium sulphate	1	7
8	3 ,, ,, ,,	1	9
9	5 ,, "super"	1	5
10	10 ,, ,, ...	1	6
11	2 ,, kainit	1	5
12	4 ,, ,, ...	1	4
13	None. Soil sterilised	1	7
14	,, ,, ,, ...	1	7

At first it was thought that only two or three of the Little Joss cultures showed any sign of disease, but a very careful scrutiny revealed

minute traces, perhaps three or four unbroken pustules, on each culture except No. 1. It was noted that these pustules always appeared on leaves which were almost dead, and which therefore apparently had not quite the same power of resisting the disease that a leaf in full vigour possesses. This wheat then was so nearly immune to the disease under all the conditions of the experiment that no difference was perceptible between the various cultures.

The differences between the susceptibility of the various cultures of Michigan Bronze were not nearly so striking as they were in the experiment where mildew was used, but the marks in Table IV show that some differences existed. Nos. 11 and 12, receiving potash, were clearly the least diseased, though 9 and 10 which were treated with sodium phosphate were nearly as free from disease. All the cultures which received nitrogenous dressings were about equally diseased, except No. 8 which was considerably worse than any other. The cultures in the sterilised soil showed more disease than the control plants and were about equal to most of those to which nitrogen was given.

It was thought desirable to compare the preceding results with those obtained on a larger scale, and we were enabled to examine the field-plots on the farm of the Royal Agricultural Society at Woburn for this purpose. The pot-cultures at Woburn were also examined and gave some interesting results, though the treatments they had been receiving were quite different from those applied to any of our own cultures.

With regard to the pot-cultures, it was found that rust was practically absent, the amount present being so small that no idea of the susceptibility of the various cultures could be gained.

Table V shows the marks denoting the amount of mildew on the various wheat cultures and their mode of treatment.

It is seen from the above table that the severity of the attack on the control plants varied between 2 and 5 with an average of 3 to 4. Only one of the cultures receiving basic slag was marked outside this range, while the average marks were about the same: the addition of basic slag seems therefore to have had no effect on the susceptibility of the plants to mildew. The plants grown in the soil containing magnesia showed a distinct increase of susceptibility to the disease (8) which was very little, if at all, decreased by the addition of lime. These plants were much greener and less mature than those of most of the cultures, which were beginning to ripen. It is possible that an increased

susceptibility to the disease may be a result of the treatment delaying the growth of the plant: or the increased infection may be due to a rapid and vigorous growth of the plant late in the season following the check received in its young stages. It will be found that these conditions had occurred in the case of some of the other badly diseased plants.

TABLE V.

Cultures	Treatment	Mildew
38 and 41	Control	4, 5
42—47	Basic slag added in various ways ...	5, 4, 4, 3, 4
56—63 and 68	,, ,, ,, ,, ...	4, 4, 2, 4, 3, 3, 3, 4, 2
80—86, 96—100	,, ,, ,, ,, ...	3, 1, 3, 4, 4, 3, 5, 4, 2, 2, 4, 4
50	Soil containing MgO	8
51—55	,, ,, ,, (CaO added) ...	8, 7, 7, 7, 7
101 and 102	Control	3, 3
102—108	·003—·001 % Lithium phosphate ...	0, 0, 0, 0, 1, 1
109—114	·03—·01 % Zinc phosphate	4, 4, 5, 4, 5, 4
115, 117, 119	·03—·01 % Lead ,,	3, 3, 3
121	Control	4
123, 125, 127	·003—·001 % Lithium nitrate	0, 0, 6
129—134	·03—·01 % Zinc nitrate	9, 10, 10, 10, 10, 10
135—140	·03—·01 % Lead ,,	7, 7, 7, 5, 5, 5
141 and 142	Control	2, 2
143—148	·003—·001 % Lithium carbonate ...	0, 0, 1, 0, 1, 1
149—154	·03—·01 % Zinc carbonate	3, 3, 3, 1, 3, 2
155—160	·03—·01 % Lead ,,	3, 1, 3, 1, 1, 1

Turning now to the cultures to which lithium salts were applied a very marked beneficial effect was found: except in one case, where strange to say the degree of mildew was 6, the amount of disease was reduced to 1, or more often the disease was entirely absent. All the lithium salts used appeared to be equally effective in preventing the appearance of the disease, and in nearly every case an increase in the amount of the salt supplied produced an increased immunity.

The cultures treated with zinc salts showed interesting differences: those receiving zinc carbonate were slightly below the normal in the amount of disease, the phosphate increased the susceptibility slightly, while on those plants treated with zinc nitrate the disease was extremely bad and had almost killed the plants. The amount of zinc salt applied did not affect the amount of disease present. The plants treated with zinc nitrate were much shorter and less mature than other plants, and were said to have been very severely checked in the early stages of their growth; so it is possible that this, rather than any direct action of the zinc nitrate, may have been the cause of the increased disease attack.

The lead salts also varied in their effects on the disease. The plants receiving the phosphate scarcely differed from the normal; the nitrate increased the disease distinctly but not as much as the zinc nitrate did; and the carbonate decreased the attack slightly. The amount of lead nitrate added produced corresponding variations in the amount of disease present. The carbonates of all the above metals seemed to diminish the susceptibility of the wheat slightly.

The field plots of wheat were unfortunately not examined until late in the season when the wheat was almost ripe; therefore in many cases it was extremely difficult to determine how much disease there had been, especially as regards mildew. Table VI shows the treatment the various plots had received and the amount of mildew and rust on them: where no figure is given for the mildew the wheat was so ripe that no trustworthy figures could be obtained.

TABLE VI.

Plot	Manures applied annually per acre	Mildew.	Rust
1	Unmanured	1	1
2 *a*	Sulphate of ammonia (=25 lbs. NH_3)	—	—
2 *aa*	As 2 *a* with 5 cwt. lime 1905, 1909, 1910, 1911	4	4
2 *b*	,, ,, 2 tons lime 1897	4	4
2 *bb*	,, ,, ,, ,, 1897, 1905	6	5
3 *a*	Nitrate of soda (=50 lbs. NH_3)	8	5
3 *b*	,, ,, (=25 lbs. NH_3)	10	6
4	Minerals (3 cwt. "super" and ½ cwt. K_2SO_4)	0	0
5 *a*	,, and ammonium sulphate (=25 lbs. NH_3)	2	4
5 *b*	As 5 *a* with 1 ton lime 1905	3	4
6	Minerals and sodium nitrate (=25 lbs. NH_3)		3
7	Unmanured	1	1
8 *a*	Minerals and in alternate years ammonium sulphate (=50 lbs. NH_3) (1911)		6
8 *aa*	As 8 *a* with 10 cwt. lime 1905		7
8 *b*	Minerals and in alternate years ammonium sulphate (1912)		6
8 *bb*	As 8 *b* with 10 cwt. lime 1905		8
9 *a*	Minerals and in alternate years sodium nitrate (=50 lbs. NH_3) (1911)		4
9 *b*	Minerals and in alternate years sodium nitrate (1912)		4
10 *a*	"Super" 3 cwt. and sodium nitrate (=25 lbs. NH_3)		2
10 *b*	Rape dust (=25 lbs. NH_3)		4
11 *a*	K_2SO_4 1 cwt. and sodium nitrate (=25 lbs. NH_3)		2
11 *b*	Farmyard manure (=100 lbs. NH_3)		4

It is perhaps unnecessary to explain that these plots have been growing wheat and have received the same treatment continuously for over thirty years. The wheat grown this year was Square Head's Master.

From the figures available for the mildew it is seen that the unmanured plots 1 and 7, and plot 4 which receives mineral manures only, were practically free from mildew. The crops on these plots were, of course, very thin and short. The ammonium sulphate plots 2*aa*, 2*b* and 2*bb* showed a medium amount of mildew, but the plots on which the disease was worst were Nos. 3*a* and 3*b* which receive only nitrate of soda. The plants here were of only medium size, not at all luxuriant in their foliage.

The figures for the mildew cannot be considered to be at all accurate, but the two extremes were clearly enough marked, 1, 7 and 4 on the one hand and 3*a* and 3*b* on the other.

The marks for the rust are more to be depended on, and on examining them it is first noticed that the same plots 1, 7 and 4 were again almost disease-free. From the rest of the figures very little is to be gathered: on plots 8*a* to 8*bb* the addition of minerals to the ammonium sulphate seems to have increased the tendency to disease as compared with plots 2*aa* to 2*bb* which received ammonium sulphate without mineral manures: on the other hand no increased disease was noted on plots 5*a* and 5*b*. Addition of minerals to sodium nitrate on the contrary seems to have decreased the amount of disease, as may be seen by comparing plots 6, 9*a*, 9*b*, 10*a* and 11*a* with plots 3*a* and 3*b*. The dunged plot showed about an average amount of rust.

The field plots of barley were much less advanced than the wheat and showed the different degrees to which they had been attacked by mildew very plainly. On two or three of the plots a small amount of *Puccinia graminis* was found, but it was not at all common so no marks were given for the rust attack. The barley plots have received almost exactly parallel treatment to that of the wheat plots except that plots 2*b*, 5*b*, 8*aa* and 8*bb* were given another dressing of lime this year: also 5*a* is divided into 5*a* and 5*aa*, the latter having received a dressing of lime in 1905. The variety of barley grown this year was Goldthorpe. The marks assigned for the amount of mildew on the barley plots are given in Table VII.

Plots 1 and 4, as we should have expected from our previous experience, were two of the least diseased; but it is at first surprising to find that No. 9*a*, which received a large dressing of nitrate of soda the previous year in addition to minerals, showed so little disease. In all probability the soluble nitrates had been completely washed out of the porous Woburn soil. The small amount of mildew on the dunged plot is also rather strange, especially as the plants were growing

luxuriantly. The disease was worst on plots 3*a* and 3*b* which received sodium nitrate only, and then on the 8's, which were manured with minerals and ammonium sulphate. The 8's are worse than the 2's which received ammonium sulphate only; but the 5's which also received ammonium sulphate and minerals were only slightly worse than the average. Plots 6 and 9*b*, which were both dressed with sodium nitrate and minerals, showed about the average amount of disease. Where plots received a nitrogenous dressing only in alternate years it was seen that the mildew was worse on the plots which received a dressing this year than on those which were manured in the previous year: cf. 9*a* and 9*b*; 8*aa* and 8*bb*.

TABLE VII.

No.	Manures applied annually per acre	Extent of mildew
1	Unmanured	3
2*a*	Sulphate of ammonia (=25 lbs. NH_3)	—*
2*aa*	As 2*a*, with 5 cwt. lime, 1905, 1909, 1910, 1912	6
2*b*	,, ,, 2 tons lime, 1897, 1912	8
2*bb*	,, ,, ,, ,, 1897, 1905, 1912	6
3*a*	Nitrate of soda (=50 lbs. NH_3)	10
3*b*	,, ,, (=25 lbs. NH_3)	8
4	Mineral manures (3 cwt. "super" and ½ cwt. K_2SO_4)	3
5*a*	,, ,, and sulphate of ammonia (=25 lbs. NH_3)	—†
5*aa*	As 5*a*, with 1 ton lime 1905	6
5*b*	,, ,, 2 tons lime 1897, 1912	7
6	Mineral manures and nitrate of soda (=25 lbs. NH_3)	5
7	Unmanured	5
8*a*	Mineral manures, and in alternate years, sulphate of ammonia (=50 lbs. NH_3) (1911)	—†
8*aa*	As 8*a*, with 2 tons lime 1897, 1912	8
8*b*	Mineral manures, and in alternate years, sulphate of ammonia (=50 lbs. NH_3) (1912)	—†
8*bb*	As 8*b*, with 2 tons lime 1897, 1912	9
9*a*	Mineral manures, and in alternate years, nitrate of soda (=50 lbs. NH_3) (1911)	3
9*b*	Mineral manures, and in alternate years, nitrate of soda (=50 lbs. NH_3) (1912)	6
10*a*	"Super" 3 cwt. and nitrate of soda (=25 lbs. NH_3)	5
10*b*	Rape dust (=25 lbs. NH_3)	5
11*a*	K_2SO_4 1 cwt. and nitrate of soda (=25 lbs. NH_3)	5
11*b*	Farmyard manure (=100 lbs. NH_3)	1

* No crop. † Observations accidentally omitted.

On the whole perhaps the field plots do not show such decisive results as was expected from a consideration of the water- and pot-cultures. But it must be remembered that in dealing with such plots which have received the same treatment for many years other factors come into play besides the direct effect of the manure on the plant.

Some of the irregularities in the results may be brought about by the different chemical and physical conditions existing in the soils of the various plots as a result of the continuous abnormal treatments they have received. Thus the immediate effect of the manure on the plant is possibly counterbalanced by some of these other factors which cannot be estimated.

The conclusions which can at present be drawn from these investigations may be shortly summarised as follows:

Susceptibility to mildew and yellow rust in wheat, and to mildew in barley, is increased by providing the plants with large amounts of available nitrogen: ammonium sulphate and sodium nitrate seem to be equally effective in this direction.

Mineral manures, especially potash salts, on the contrary decrease the susceptibility to disease but cannot counteract the effect of large quantities of nitrogenous manures.

Plants which are semi-starved as regards nitrogen exhibit a considerable degree of immunity, even if the phosphates and potash are also present only in small quantities.

Lithium salts are also effective in producing immunity, while nitrates of lead and zinc, particularly the latter, render plants extremely susceptible. Other salts of lead and zinc have very little effect on the susceptibility of plants.

A variety of wheat which is almost immune to a disease (such as Little Joss to yellow rust) tends to retain its immunity even when supplied with excess of nitrogenous food-material.

Increased immunity does not appear to be due to a lack of food-material available for the fungus in the host, as suggested by M. Ward, because the plants rendered relatively immune by adding phosphates or potash to their food-supply were as healthy and well-grown as those receiving no such additions.

It yet remains to be seen what physiological explanation can be found to account for the changes in susceptibility which can be produced in plants by the above means.

ON THE GROWTH OF PLANTS IN PARTIALLY STERILISED SOILS.

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During the past few years we have grown a large number of plants in partially sterilised soils alongside of others in untreated soils, in order to compare the total weights of dry matter produced. While the experiments were in progress certain qualitative differences in growth and habits of the plants revealed themselves; we propose in this paper to record these differences and to set out certain of the hypotheses that have been put forward to account for them. It was soon found that several distinct problems in plant physiology were concerned, and that any attempt to test the hypotheses experimentally would involve us in a number of side issues and lead us away from our main purpose. We therefore for the present confine ourselves to a statement of the facts observed, leaving their more complete elucidation for later work.

Partial sterilisation is effected in our laboratory in three ways: by treatment with volatile or easily decomposable antiseptics which are subsequently removed, by heating to a temperature just sufficient to put out of action the factor detrimental to bacteria (about 55° C.), and by heating to a higher temperature (100° C.)[1].

When seeds are placed in soils so treated it is at once observed that their rate of germination has been affected. Germination is sometimes more and sometimes less rapid than in untreated soils under similar conditions of temperature and moisture, but the exact amount of the acceleration or retardation varies very much with the

[1] The temperatures throughout are ° C.

soil, the seed, the amount of water, etc. Retardation is almost always produced in soils heated to 100° or treated with toluene, but acceleration is often obtained in soils heated to 55° C. Some seeds, however, such as the tomato, are almost always retarded in germination. The effect is generally more pronounced in a moist than in a dryer soil, and more in a rich than in a poor soil. Accelerated germination when it occurs is usually shown only by a certain number of the seeds and not by all, whereas retarded germination may in some cases be shown by all the seeds.* Generally speaking the most marked effects are obtained in soils heated to 100°, the next in soils treated with toluene, and the least in soils heated to 55°, but there is a distinct qualitative difference between soils treated with toluene, and those heated to 55°, in that accelerated germination occurs more frequently in the latter, as already stated. These effects are produced in all the soils examined though with marked differences in degree. They are seen only to a comparatively small extent in ordinary arable soils and may indeed require careful experiments to detect them, but they are very obvious in heavily manured or pasture soils.

Turning now to the seedling stage: the differences in the plant depend to a large extent on the soil. In relatively poor soils the plants on the partially sterilised soils are sometimes indistinguishable from those on the untreated soil and sometimes larger in size but otherwise similar. In rich soils marked differences occur, which vary with the method of sterilisation but are most striking in soils heated to 100° C. Seedlings growing on the heated soils have, in comparison with those growing on untreated soils, smaller roots and smaller cotyledons of a darker green colour frequently showing some purple. The cotyledons of tomatoes find a difficulty in shedding their seed cases and spreading out, and they also tend to curl under instead of lying flat (Fig. 2).

When the second leaves appear a new set of differences come in which we have noticed particularly in the case of tomatoes grown in pots.

The young tomato plant often shows a considerable amount of purple pigment, especially on the lower part of the stem and the under surface of the leaves. When this appears growth becomes very slow, so that the plant is considerably stunted in comparison with others free from the pigment. The phenomenon is well known to commercial growers who speak of it as a "hard growth" in contradistinction to the soft, sappy growth of a green plant that has suffered no check or

stunting during its life time. It appears that the purple colour is confined to the epidermis, for on stripping this off the normal green appears.

Some of the conditions necessary for the formation of the purple pigment are associated with the soil, and we find it more frequently in steamed than in untreated soils. Thus, when plants on the untreated soil are of a normal green colour, those on the soil heated to 100° may have purple stems and leaves of a dirty green or very dark colour on the upper surface, and deep purple on the veins and the under surface, this being particularly true of the young leaves. So long as the purple persists, growth is considerably retarded and in consequence the plants on the heated soil grow much more slowly than those on the untreated soil.

These effects are very marked in the dull days of winter. A little later on a striking change sets in. The purple colour rapidly disappears, and forthwith the plant begins to make most remarkable growth. Its leaves still remain darker in colour than those of the plants in the untreated soil, but the colour is now a true green untinged with purple. Every part of the plant grows rapidly (Fig. 3). An astonishing development of fibrous root takes place, far exceeding anything produced on untreated soils: the actual weight of root, however, is not necessarily large (Table VII). The stem becomes very stout and the leaves are large and of great substance. Frequently the nodes shorten so that the plant becomes denser and more compact; this, however, depends somewhat on the plant, occurring invariably with cereals[1] and chrysanthemums, but not with tomatoes. Before long the plant is far heavier than those on the untreated soil. As the plants mature the leaves keep green longer and do not so soon take on their red or yellow colours; the bottom leaves also stay on longer than is the case with plants on untreated soils.

But this vigorous vegetative growth does not cause the fruit to suffer. Our tomato plants in heated soil not only flowered earlier, but produced more flowers, more fruit, and earlier and sweeter fruit; they also lasted longer and fruited longer than those in the untreated soil. Earlier flowers and fruit were also obtained from cucumbers, and earlier and larger flowers of lighter colour from chrysanthemums, when these plants were grown in partially sterilised soils.

It commonly happens in late spring and summer, when the light is good and conditions are favourable to plant growth, that the

[1] Measurements for rye are given in this *Journal*, 1909, **3**, 122.

retardation does not appear at all, but the plants go ahead right from the start. We have not yet discovered the precise conditions under which this takes place, and we can never predict with certainty whether retardation or acceleration of growth will set in during the first few weeks in heated soil. Apart from its physiological interest the retardation is of great importance in the practical applications of partial sterilisation because of its frequent occurrence in the early season when the grower under glass is trying to push his plants ahead as quickly as possible. We cannot too strongly emphasise the fact that these remarkable and apparently interchangeable accelerations and retardations *are only met with in the early stages of plant growth.* Later on the rate of growth is governed by the rate of production of ammonia and nitrate in the soil.

If the plants are cut and analysed at any time during the period of active growth those on the heated soil are found to contain a higher percentage of nitrogen and sometimes of phosphoric acid, and the difference extends also to the fruit. When growth is ended and the plant is dead the translocation of nitrogen, phosphorus and potassium from the root and stem to the fruit is commonly found to have been more complete on the heated than on the untreated soil.

With some modifications in detail the same kind of results are obtained with all other plants that we have tried. Mustard is much retarded in growth during its early days in heated rich soil, and takes on a very dark green unhealthy colour. We have not observed the purple patches so prominent in tomato plants, but instead the leaves develope a remarkable tendency to curl towards the under surface; so long as this happens growth is retarded. But later on the leaves open out and vigorous growth takes place, so that the plants soon surpass those on the untreated soil, and, as in the case of tomatoes, they contain a higher percentage of nitrogen, and sometimes of phosphoric acid, in their dry matter. Grasses take on a deep green colour and are commonly retarded in growth but their leaves did not in our experiments show the peculiar curling.

Chrysanthemum cuttings do not generally "strike" as readily in heated as in untreated soil, there being a marked delay, as before, in the formation of root. But once root development begins it continues rapidly till after a time the usual fibrous mass of root is formed (Fig. 4).

Soil heated to 55° behaves entirely differently as a medium for plant growth. The retarded stage is either not induced or is of very brief

duration and the plants rapidly surpass those on the untreated soil and on the soil heated to 100° (Fig. 5). Sometimes growth is particularly rapid giving rise to plants which are extraordinarily large considering their early stage (Fig. 6). There is no characteristic appearance associated with the plants and, except for their larger size, their rather earlier flowering, and more prolific fruiting, they are indistinguishable from those on the untreated soil, and show none of the peculiarities of the plants on the soil heated to 100°. They do not make the sudden late growth seen on soils heated to 100°, nor do they even maintain the rapid growth of their early days, and in the end they are inferior in weight to plants grown on steamed soils. The amount of growth, as before, is conditioned by the amount of ammonia and nitrate made.

Soils treated with toluene sometimes behave qualitatively very much like soils heated to 55°, but on rich soils retardation may be induced in the early stages. In such cases the cotyledons are smaller than usual, dark green in colour and the edges show a strong tendency to curl towards the under surface. The final growth may be greater or less than on soils heated to 55°, but is always less than on soils heated to 100°.

Other volatile antiseptics behave like toluene.

In all these cases the plants contain a higher percentage of nitrogen and sometimes of phosphoric acid in their dry matter than those on untreated soils; they also generally show more complete translocation to the fruit.

Both phenomena are less marked than in soils heated to 100°.

A careful study of the preceding observations brings out seven important directions in which partially sterilised soils differ from untreated soils:

1. There is generally a retardation in germination but sometimes partial acceleration (*i.e.* affecting some of the seeds only).

2. There is generally an acceleration in growth up to the time of the appearance of the 3rd or 4th leaves, but sometimes a marked retardation, especially in rich soils heated to 100° C. We have failed to discover the conditions regulating the retardation and can never predict with certainty whether or not it will set in. On the whole we have observed it more frequently during dull winter days than in the brighter spring or summer days.

3. When this retardation occurs it is accompanied by a very dark green leaf colour and either the formation of a purple pigment or a tendency for the leaves to curl towards the under side. The whole appearance is strongly suggestive of an attempt on the part of the plant to reduce assimilation.

4. Later on the purple colour goes and the curling ceases; rapid plant growth then takes place. The subsequent growth is finally proportional to the amount of food present.

5. Plants grown in soils heated to 100° show a very remarkable development of fibrous root unlike anything obtained on untreated soils.

6. Plants grown on soils heated to 100° have, in comparison with those on untreated soils, larger leaves of deeper green colour, stouter stems, usually shorter internodes; they flower earlier and more abundantly, and contain a higher percentage of nitrogen and sometimes of phosphoric acid in their dry matter; the roots and stems give up their nitrogen, phosphorus, and potassium more completely to the fruit.

7. Plants grown on soils heated to 55° or treated with volatile antiseptics show fewer of these effects; there is only rarely a retardation in seedling growth but usually an acceleration, sometimes a rapid one, succeeded by a period of steady growth which is finally proportional to the amount of plant food present. No specially marked development of fibrous root or shortening of the internodes occurs, but there is an increase in the percentage of nitrogen and sometimes of phosphoric acid in the dry matter as compared with plants raised on untreated soils, and also a more complete translocation of these materials to the fruit.

Two of these effects are confined to soils heated to 100°, viz. the marked development of fibrous root, and the shortening of the internodes.

The other effects are more general, viz. alteration (either retardation or acceleration) in the rate of germination and early growth; steady growth of the older plant proportionately to the amount of plant food produced in the soil; earlier and more prolific flowering and fruiting (Figs. 1 and 7); increase in the percentage of nitrogen and phosphoric acid in the dry matter, and more complete translocation of these constituents and of potassium from the root and stems to the fruit.

We may now turn to the chemical differences in the soils.

Partially sterilised soils are characterised by the fact that ammonia accumulates unchanged and nitrates are no longer formed (the nitrifying organisms being killed). Untreated soils contain practically no

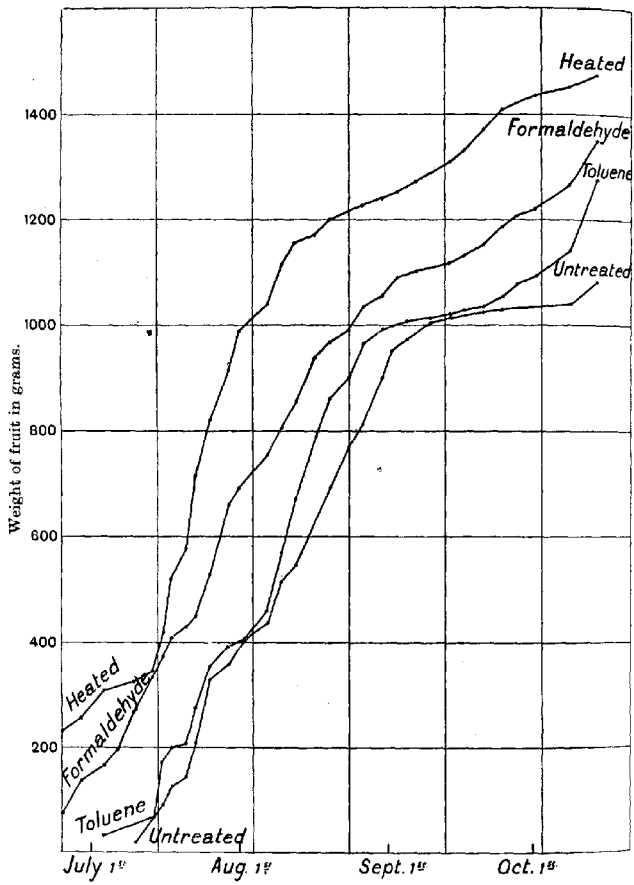


Fig. 1. Showing the rate of fruiting of tomatoes in variously treated soils.

ammonia. As the decomposition processes are all accelerated, ammonia and its antecedent bodies are present in partially sterilised soils in greater amount than the nitrates and antecedent bodies in

untreated soils. Thus the aqueous extract of partially sterilised soils is richer in nitrogen, in ammonia, and in the so-called "albuminoid ammonia" than that of untreated soils.

Soils heated to 100° C. are further characterised by the presence of decomposition products, some of which are soluble and give a brown colour to an aqueous extract, while others have a peculiar odour recalling that of liquorice. Some of the products are specially suited for the nutrition of certain moulds[1], especially *Pyronema glaucum* which often developes to a remarkable extent; and others are very unsuited for the development of certain bacteria[2]. None of these products has yet been identified in our soils[3]. Soils that have been heated to 100° appear to become more moist than untreated soils when both receive the same amount of water.

Discussion of the observations.

Several hypotheses have been put forward to account for these results, at least two of which are of considerable importance.

Germination. S. U. Pickering was the first to observe that germination is retarded in partially sterilised soils. His work arose out of an observation that young fruit trees started much more slowly into growth in heated than in untreated soils[4], although later on they made considerably better growth. As this particular problem appeared likely to require a long while for investigation he began by working on germination.

In his first paper, published in 1908[5], he states that germination is adversely affected in soil which has been heated without drying to temperatures from 60° to 150°; the total number of seeds that germinate decreases in most cases, and the time necessary for germination increases, with the temperature of heating. The result is not due to any change

[1] This fact is curiously overlooked by mycologists. We read, for instance, in a recent paper by a well-known mycologist that a fungus known to attack the roots of certain plants was inoculated into soil heated above 100° C. in order to ascertain whether it could live and thrive in a soil free from living plants. Good growth was obtained, as we should expect, but the wholly erroneous conclusion was drawn that the fungus "is able to live in, and to penetrate for some distance into, the ordinary soil in our fields."

[2] This *Journal*, 1909, **3**, 135, and 1912, **5**. 200.

[3] For an account of the American work on this subject see Schreiner and Lathrop, "The chemistry of steam heated soils," *Bul.* 89, *Bureau of Soils*, 1912.

[4] *Nature*, June 6th, 1907.

[5] "Studies in germination and plant growth." This *Journal*, 1908, **2**, 411—434.

in the bacterial flora[1]. An increase in the soluble constituents of the soil, especially the organic matter and soluble nitrogen compounds, was found to result from the heating, and the increase was considered to be directly proportional to the increase in time required for germination. "The latter increase appears, therefore, to be due to the formation of a nitrogenous compound in the soil, which is inhibitory towards germination." The premises were subsequently modified somewhat, the direct proportion being found not to hold[2], he therefore supposes "either that the inhibiting substance produced by heating different soils is not the same in all cases, or, more probably, that it constitutes only one of the organic products formed when a soil is heated, the proportions between it and the other non-inhibiting substances formed varying in each particular case."

In this second paper he showed that soils treated with antiseptics also adversely affected germination and behaved like soils heated to 60°—75°. He further states that "soils in their natural state appear generally to contain a certain amount of this inhibitory substance."

A third paper[3] is devoted to a study of the properties of the soluble matter in soils, and the conclusion is drawn that the amount of soluble organic matter, and the toxic qualities of heated soils, are reduced on storing the soils under moist, aerated conditions.

There is no doubt that heat causes decomposition in the soil, the amount of which increases as the temperature of heating rises. Pickering inclines to the belief that the change is regular and progressive: our own experiments, however, indicate that the decomposition going on above 80° is far more serious than that taking place at lower temperatures. It is equally certain that something is produced harmful to germination and, as we have shown elsewhere (see p. 255), to bacterial activity. Since the water extracts of the variously treated soils cause accelerations and retardations in germination of the same order as is shown by the soils themselves it follows that the active substances are partly, at any rate, soluble in water.

[1] These observations were made on *Lolium perenne*, *L. italicum*, clover and spinach, and are of interest in connection with a hypothesis, attributed to Nilson, that germination depends on the activity of bacteria at the surface of the seed. W. Windisch and K. Schönewald (*Woch. Brau.* 1905, **22**, 200) showed that this was not true of barley, and Dixon (*Trans. Roy. Soc. Dub.* v. **11**, 1) that it did not hold for turnips. Hutchinson and Miller have also germinated peas and wheat in absence of bacteria (This *Journal*, 1912, **4**, 282—302). R. W. Stigell has discussed changes whereby bacteria may affect germination and subsequent plant growth (*Centr. Bakt. Par.* 1909, **23**, 727).

[2] "The action of heat and antiseptics on soils." This *Journal*, 1908, **3**, 32—54.

[3] "Studies of the changes occurring in heated soils." This *Journal*, 1910, **3**, 258—276.

But there is no evidence that these active substances are necessarily organic. Even if the supposed proportionality between the amount of soluble nitrogen and organic matter on the one hand, and the retardation of germination on the other, had been confirmed, the proof is inconclusive because the conditions would be fulfilled by ammonia just as well as by organic matter. Now that the proportionality is found not to be general the argument in favour of the invariable organic nature of the retarding agent loses its force.

Unfortunately the soil extract, like the soil itself, is very complex in composition and not readily resolved into its constituents, so that recourse cannot be had to the simple expedient of trying the effect of each constituent on germination. It is certain, however, that ammonia and nitrates are present, and we have made some experiments to ascertain what part they play in the matter.

Very dilute solutions of ammonium hydrate (one part per million of the medium) were found to accelerate germination under the conditions of our experiments. Stronger solutions had no effect, still stronger solutions retarded the process, and finally at higher concentrations germination was prevented altogether. The details of the effect vary considerably with the conditions, but the experiments show that when, for any reason, germination is slow, very dilute ammonia solutions tend to accelerate it; when germination is more rapid, ammonia tends to retard it.

This result is probably connected with another obtained by A. J. Brown[1]. If barley seed is placed in solutions of various substances, water alone enters the seed and not the dissolved substance as a rule. There are, however, certain substances that can penetrate the membrane[2], among them ammonia, and in this case water enters the seed more rapidly from the solution than from pure water. It seems likely that this increased speed of entry of the water into the seed is in part responsible for the acceleration induced by very dilute ammonia solutions in the germination of slowly germinating seeds. The fact also that ammonia enters the seed probably accounts for its very drastic effects at greater concentrations.

Ammonium sulphate solutions are much weaker in their action on germination and sodium nitrate solutions weaker still at equivalent concentrations.

[1] *Proc. Roy. Soc.* 1909, **81** B, 82—93. The selective permeability of the coverings of the seeds of *Hordeum vulgare*.

[2] These are the "hormones" of H. E. and E. F. Armstrong, see p. 260.

It may be taken as certain that all the soluble constituents of the soil have an effect on germination. They probably fall into at least two groups, some behaving like ammonia and causing very great retardation at higher concentrations, while others like sodium nitrate have much less effect. Further experiments would be necessary to decide whether all the powerful retarders would accelerate in dilute solutions as ammonia does. F. J. Seaver and E. D. Clark[1] have argued that the decomposition products are not actually toxic to higher plants, but are harmful only by reason of their excess. They make the important point that, as *Pyronema* and other moulds will grow on heated soils more readily than on unheated soils, it is obviously unsound to speak generally of toxins in heated soils. But so long as we confine ourselves to the higher plants we think we are safe in making the distinction given above.

Our experiments indicate that germination is too sensitive a process, and too susceptible to external influences, to afford much help in studying the soil. It is greatly affected by small changes in moisture content, ammonia content, temperature, etc., and is more useful in detecting such changes than in studying them. We do not, therefore, agree that the retardation of germination affords any proof of the formation of any particular organic toxin, but consider that it may result from any change in the soluble constituents; some substances, including ammonia and doubtless certain organic compounds, have a very marked effect, while others, such as nitrates, have a smaller action.

This conclusion also harmonises with most of Pickering's experimental results. The main discrepancies between his results and ours are that (1) we find accelerated germination in certain cases in soils heated to 55°, (2) we failed to trace any proportionality between the analytical data for the soil and the amount of retardation or acceleration in germination[2]. The effect produced depended so much on the moisture, the temperature, and other conditions of the experiments, and also on the individuality of the seeds, that we could not express it by any number; nor could we even find any general connection between the nature of the effect and the amount of nitrate or ammonia in the soil.

[1] "Biochemical studies on soils subjected to dry heat," *Biochem. Bull.* 1912, **1**, 413–427.

[2] It should be noted that we are not dealing with highly heated soils as used in some of Pickering's experiments but confined ourselves to soils treated with antiseptics or heated to 100° C. or less. When the temperature of heating rises above 100° the decomposition proceeds much more rapidly and the effect on the plant is correspondingly more drastic.

Lastly, we could obtain no definite proof that the harmful effect of heated soil on germinating seeds passes off after a time. The difference in behaviour between soil heated to 100° and untreated soil apparently becomes reduced on storage, but the untreated soil itself was found to change in composition and behaviour towards germinating seeds. No unchanging standard could be discovered for making any strict comparison between freshly treated and stored soils.

The seedling stage and early growth. Some interesting physiological problems are presented by the subsequent effects shown during the seedling stage and up to the formation of the third or fourth leaves. The curling of the leaf towards the under side, the very dark green colour and the purple patches all tend apparently to reduce the amount of assimilation, but it is not at all obvious why this should be necessary to plants growing in treated soil where there is so much plant food present. Somewhat similar phenomena can be induced by restricting the supply of potassium and giving excess of nitrogen; the young plants in several ways resemble the mangolds on Plot 5 AC of Barnfield where large dressings of ammonium sulphate and rape cake are given, but no potassium salts. (In this case there is no purple colour but the leaf stems are orange instead of the normal pale green.) Further, the effect does not depend entirely on any soil constituent, but requires also certain external conditions not definitely ascertained, of which poor light appears to be one.

For the present we prefer not to discuss the immediate causes of these phenomena but to confine ourselves to another remarkable relationship: the close connection between the pigmented or stunted leaves and small growth on the one hand, and the large green leaf and rapid growth on the other. As already stated, we can never be quite certain how *young* plants will behave in partially sterilised soils; they may either grow much more rapidly than those on untreated soils, throwing out larger cotyledon leaves and larger subsequent leaves, or they may show the remarkable pigmented or curling effects and considerable retardation of growth. These two sets of phenomena are very closely connected and it would be quite reasonable to suppose that they are both produced by the same factors, either retardation or acceleration setting in according as some small change just shifts the balance one way or the other.

It was at one time considered that any substance toxic to plants may act as a stimulant if supplied in sufficiently minute quantities, and on this view it is only necessary to suppose that partial sterilisation

leads to the formation of a toxin which is sometimes in such small amounts that it stimulates and sometimes in larger amounts so that it retards growth. But it is now known that so typical a toxin as copper sulphate retards growth and never increases it in any well conducted experiment. This view must therefore be regarded as too vague to be helpful. A much more definite conception has been put forward by H. E. and E. F. Armstrong[1]. Certain substances which usually have but a slight attraction for water are capable of penetrating plant membranes and entering the cells: instances are toluene, carbon disulphide, chloroform, etc. Once there they are considered to stimulate enzymic activity, and exercise a determining influence in regulating metabolism; one effect, for instance, is to condition the introduction of water which dilutes the cell contents and thus determines the occurrence of downgrade changes. The authors further suggest that the absorption of salts by plants, and the translocation of diffusible material, may be largely determined by some such process. To these bodies the name hormone is given; they are not a purely arbitrary group but possess certain chemical and physical properties which more or less differentiate them from others.

Now ammonia occurs in the list of hormones and is described as acting very rapidly. We have seen that it behaves in a special manner towards the germination of seeds, accelerating the process in very dilute solutions (the typical hormone action) and considerably retarding it in rather stronger solutions, the action being very much more marked than that shown by solutions of salts, even of ammonium sulphate. Ammonia also occurs in all the soils, especially in those that have been partially sterilised. Unfortunately we have no means of knowing precisely in what state it occurs, but some at any rate is apparently free. In view of its special behaviour towards the cell membranes we have no right to regard it solely as a nutrient but must consider the possibility of other effects. It would not be difficult to sketch out a hypothesis to account for the retardation and acceleration results on the assumption that the ammonia in the soil acts as a hormone.

Our own data are insufficient to settle the problem because the partially sterilised soil differs in another way from the untreated soil; it contains a different set of nutrients; there are thus two variables.

Pickering has shown that soils heated to 125° and 130° C.[2] are

[1] "The origin of Osmotic effects, III." *Proc. Roy. Soc.* 1910, **82** B, 588—602. "The function of hormones in regulating metabolism." *Annals of Botany*, 1911, **25**, 507—519.

[2] "Plant growth in heated soils." This *Journal*, 1910, **3**, 277—284.

less favourable to the growth of spinach, tomato, and tobacco than soils heated to 100° C. only, and concludes that at the higher temperature a larger production of toxin has taken place. We have repeated the experiment with tomatoes and obtained a like result. Since the second crop is not adversely affected he supposes that the toxin is unstable and gradually disappears by the action of air and moisture. The supposition is unnecessary: in so far as ammonia is responsible for the retardation its effect in a cropped soil must steadily diminish as it is absorbed by the plant. A second crop in any case is less likely to suffer than an early sown first crop: all our observations show that the retarding effects are less marked in summer than in early spring.

The marked development of fibrous root in the soil heated to 100° C. is being further studied; it occurs in a top dressing of heated soil added to a border of untreated soil, and such top dressings are being now adopted to induce fine root growth in one or two nurseries where some of our experiments have been made.

The difference in composition in dry matter—an increased percentage of nitrogen and sometimes of phosphoric acid in the case of plants grown on partially sterilised soils—seems to be associated with the differences set up in the soil. Plants fed on ammonium salts under conditions where nitrification is suspended commonly contain a higher percentage of nitrogen than plants fed in the normal manner on nitrates. The phosphoric acid relationships are being further studied. But it is probable that the altered composition of the leaves and stems and more complete translocation of nitrogen, phosphorus and potassium to the fruit are closely connected with the earlier and more prolific fruiting and with the change in quality of the fruit.

No hypothesis has yet been put forward to account for the shortening of the internodes in the plants grown in the heated soil.

EXPERIMENTAL PART.

Germination.

The effect of soil treatment on the rate of germination. The soil was passed through a 3 mm. sieve and then carefully divided into several similar portions each of which was treated in its proper manner. One was heated to 100° C. for three hours in a steam oven; another received 0·5 per cent. of toluene which at the end of 24 or 30 hours was allowed

to evaporate by spreading the soil in a thin layer, the water simultaneously lost being subsequently added; the third was heated to 55° C. for three hours in a water oven; while the fourth was left untreated. In order to study the behaviour of germinating seeds 40 gram lots of the treated and untreated soils were weighed into Petri dishes, there being usually six dishes of each of the various soils, *i.e.* 24 altogether. The same amount of water was added to each dish so that all should be uniformly moist; the soil was made distinctly wet but was not actually waterlogged. One hundred seeds (usually turnips or tomatoes) were placed on the top of the soil in each dish but not buried, and the whole of the dishes were then put into the incubator at 22° C. After germination had begun the dishes were taken from the incubator so that the number of germinated seeds might be counted. Any seed in which the radicle could be seen was regarded as having germinated. To facilitate the counting, the obviously germinated seeds were removed by an assistant from each dish and set aside, and the more doubtful ones were then examined carefully by one of us. With practice it was found possible to get through all the dishes fairly quickly.

TABLE I. *Number of seeds germinated in given periods. Turnip seed. Temperature* 18° C.

No. of dish	15 hrs.	17 hrs.	19 hrs.	21½ hrs.	26½ hrs.	39 hrs.	63 hrs.	87 hrs.	3 days later
1	6	16	46	73	83	89	92	92	98
2	5	21	46	67	78	90	95	96	100
3	5	18	45	69	80	91	95	96	99
4	5	18	44	71	81	93	98	101	105
5	6	23	54	75	81	93	99	100	102
6	6	21	50	69	78	88	92	93	100

Thus each unit in our experiment consists as a rule in six dishes of 100 seeds each, *i.e.* 600 seeds altogether. By using this large number we minimise the difficulties due to the very considerable individual variation in seeds, and reduce our experimental error to comparatively low proportions. Table I gives the detailed counts for the six untreated soils in one experiment, the probable error for the individual dishes is 1 to 2·5, and the mean error for the six is about 1. The errors for the treated soils are of the same order.

TABLE II. *Number of seeds germinating in given periods in various soils immediately after treatment.*

A. *Poor arable soil.* "Little Hoos" containing 0·12 % N, 2·10 % $CaCO_3$ and losing 5·31 % on ignition.

Turnip seed. 28. III. 11.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after					Total germinating
	Ammonia	Nitrate	Total	19 hrs.	24 hrs.	27 hrs.	39 hrs.	63 hrs.	
Untreated	1	9	10	6	44	306	435	463	497
Heated to 100° C.	4	9	13	4	61	309	421	456	490
" " 55° C.	1	9	10	8	58	315	437	471	503
Treated with Toluene	2	9	11	2	33	294	420	462	501

Exp. 115, 47.

B. *Arable soil.* "Knotwood" much richer in nitrates and containing 0·18 % N, 0·37 % $CaCO_3$, and losing 7·6 % on ignition.

Turnip seed. Moisture = 36 %. 29. XI. 11.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after				Total germinating
	Ammonia	Nitrate	Total	18½ hrs.	22½ hrs.	39½ hrs.	64½ hrs.	
Untreated	5	56	61	89	291	461	512	544
Heated to 100° C.	8	56	64	83	271	486	547	574
" " 80° C.	7	56	63	78	262	465	519	553
" " 55° C.	5	56	61	77	277	463	524	561

Exp. 143, 13.

(With less water there had been acceleration at 55° C., no action at 80° C., and retardation at 100° C.)

C. The same soil. Tomato seed. Moisture = 33 %.

Soil treatment	Seeds germinated after			
	46 hrs.	70 hrs.	95 hrs.	
Untreated	70	106	132	340 seeds in each set
Heated to 100° C.	55	90	109	
" " 80° C.	60	77	94	
" " 55° C.	62	94	115	

Exp. 143, 17.

TABLE II (*cont.*).

D. Garden soil containing 0·42 °/₀ N, 2·6 °/₀ $CaCO_3$ and losing 12·8 °/₀ on ignition. Turnip seed. Moisture = 40·7 °/₀. 20. VII. 11. Temp. 25° C.

Soil treatment	Seeds germinated after						Total germinating
	15 hrs.	17 hrs.	18½ hrs.	21 hrs.	23 hrs.	39 hrs.	
Untreated.....	49	195	303	435	460	560	592
,, ...	48	197	309	442	472	568	598
Heated to 100° C....	62	211	318	409	452	552	592
,, ,, 55° C....	63	246	358	445	473	564	586

Exp. 115, 94.

E. Turnip seed. The same soil. Moisture = 36 °/₀. 6. XII. 11.

Soil treatment	Seeds germinated after					Total germinating
	17½ hrs.	19 hrs.	23 hrs.	40 hrs.	47½ hrs.	
Untreated	51	169	369	528	542	591
Heated to 100° C.	23	128	355	519	532	595
,, ,, 80° C.	57	180	380	539	556	615
,, ,, 55° C.	89	198	375	515	528	589

Exp. 148, 21.

F. Tomato seed. Same soil. Moisture = 39 °/₀. 9. XII. 11.

Soil treatment	Seeds germinated after				Total germinating
	45 hrs.	53 hrs.	69 hrs.	76 hrs.	
Untreated	250	367	462	503	554
Heated to 100° C.	74	136	336	443	528
,, ,, 80° C.	92	149	323	463	527
,, ,, 55° C.	106	165	360	465	534

Exp. 143, 25.

G. Very rich greenhouse soil. "Ox. L." containing 0·63 °/₀ N, 1·93 °/₀ $CaCO_3$ and losing 16·9 °/₀ on ignition.
Turnip seed. Moisture = 60 °/₀. 14. XII. 11.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total	16½ hrs.	19 hrs.	23½ hrs.	40 hrs.	64 hrs.	88 hrs.	
Untreated	14	360	374	18	106	361	521	562	566	608
Heated to 100° C. ...	60	360	420	6	86	343	514	557	563	597
,, ,, 80° C. ...				11	76	324	504	555	560	598
,, ,, 55° C. ...				18	121	382	522	561	564	601

Exp. 143, 29.

TABLE II (*cont.*).

H. Tomato seed. Same soil. Moisture = 60 %. 19. XII. 11.

Soil treatment	Seeds germinated after		
	46½ hrs.	53 hrs.	
Untreated	124	202	340 seeds in each set
Heated to 100° C.[1]	24	44	
,, ,, 80° C.	21	42	
,, ,, 55° C.	84	137	

Exp. 143, 33.

The moisture is in all cases calculated on the moist soil and the percentage composition on the dry soil.

[1] In this case the soil after heating to 100° wetted more easily than the untreated soil. This is the reverse of what usually happened.

TABLE III. *Effect of storing the soil after treatment. Number of seeds germinating in given periods.*

A. Greenhouse soil. "R.C." (tomato soil) containing 0·37 % N, 0·57 % $CaCO_3$ and losing 8·7 % on ignition. Turnip seed.

1. Immediately after treatment. Moisture = 47·5 %. 12. VII. 11. Temp. 18° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total	16 hrs.	18 hrs.	20 hrs.	22½ hrs.	27½ hrs.	40 hrs.	
Untreated	10	50	60	33	117	285	424	481	544	602
Heated to 100° C. ...	38·5	50	88·5	18	99	247	380	467	530	590
,, ,, 55° C. ...	23	50	73	23	119	292	428	489	556	602
Treated with Toluene	30·5	50	80·5	27	109	270	391	460	536	595

Exp. 115, 88.

2. Two months after treatment. Moisture = 47·5 %. 12. IX. 11. Temp. 20° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after					Total germinating
	Ammonia	Nitrate	Total	14 hrs.	16½ hrs.	19½ hrs.	22½ hrs.	26 hrs.	
Untreated	7	149	156	47	196	342	424	511	543
Heated to 100° C.	85	(50)	135	36	190	345	429	532	572
,, ,, 55° C.	7	147	154	51	202	357	430	518	552
Treated with Toluene	71	95	166	59	208	358	433	517	560

Exp. 135, 8.

TABLE III (*cont.*).

3. Six months after treatment. Moisture = 47·5 %. 9. I. 12. Temp. 17° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after				Total germinating
	Ammonia	Nitrate	Total	18 hrs.	21½ hrs.	39 hrs.	63 hrs.	
Untreated	4	176	180	34	212	484	517	540
Heated to 100° C. ...	99	50	149	33	200	493	547	566
,, ,, 55° C. ...	5	167	172	36	204	509	544	559
Treated with Toluene	90	83	173	24	216	483	614	629

Exp. 135, 52.

(For results with sand containing equivalent amounts of $(NH_4)_2SO_4$ and $NaNO_3$ see Table VI, D.)

B. Tomato seed. Immediately after treatment. Same soil as for turnips in all cases. 15. VII. 11. Temp. 21° C.

Soil treatment	Seeds germinated after									Total germinating
	40 hrs.	46 hrs.	50 hrs.	63 hrs.	71 hrs.	87 hrs.	95 hrs.	111 hrs.	135 hrs.	
Untreated	18	44	71	164	205	249	254	275	285	288
Heated to 100° C.	2	3	7	40	65	142	154	213	266	300
,, ,, 55° C.	1	4	15	70	108	187	202	246	279	295
Treated with Toluene	0	0	0	33	57	132	154	205	272	290

Exp. 115, 91.

Six months after treatment. Same soil as for turnips. 9. I. 12.

Soil treatment	Seeds germinated after			Total germinating
	44 hrs.	49 hrs.	68 hrs.	
Untreated	158	297	390	400
Heated to 100° C.	69	194	385	407
,, ,, 55° C.	80	218	387	401
Treated with Toluene	82	222	385	402

Exp. 135, 62.

TABLE III (*cont.*).

C. Garden soil containing 0·42 °/₀ N, 2·6 °/₀ $CaCO_3$ and losing 12·8 °/₀ on ignition.

Turnip seed. Immediately after soil treatment. Moisture = 36 °/₀.

14. IX. 11. Temp. 22° C.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after				Total germinating
	Ammonia	Nitrate	Total	15½ hrs.	17½ hrs.	19½ hrs.	22½ hrs.	
Untreated	6	44	50	43	137	290	391	572
Heated to 100° C. ...	22	47	69	52	147	293	402	577
,, ,, 55° C. ...	15	44	59	92	214	331	415	582
Treated with Toluene	15	41	56	75	181	315	393	563

Exp. 135, 11.

This soil had been kept in bottles for two months previous to treatment.

Four months after treatment. Moisture = 40 °/₀. 16. I. 12. Turnip seed.

Soil treatment	Ammonia and Nitrate per million of dry soil			Seeds germinated after			Total germinating
	Ammonia	Nitrate	Total	18 hrs.	21 hrs.	42 hrs.	
Untreated.....	8	82	90	89	242	513	553
Heated to 100° C. ...	87	78	165	82	262	525	568
,, ,, 55° C. ...	23	105	128	80	238	525	570
Treated with Toluene	9	120	129	80	233	526	554
Sand and distilled water.....				167	314	506	558

Exp. 135, 58.

Arable soil containing 0·11 °/₀ N, 3·4 °/₀ $CaCO_3$ and losing 4·0 °/₀ on ignition.

4½ years after treatment. 11. I. 12. Turnip seed. Moisture = 40 °/₀.

Soil treatment	Seeds germinated after				Total germinating
	17½ hrs	20 hrs.	23 hrs.	41½ hrs.	
Untreated	15	66	140	199	225
Heated to 100° C.	8	52	113	187	213
Treated with Toluene	12	47	121	203	224

Exp. 135, 54.

TABLE IV. *Effect of varying soil moisture content on the rate of germination of seeds.*

A. Arable soil ("Knotwood field") containing 0·18 % N, 0·37 % $CaCO_3$ and losing 7·6 % on ignition. Turnip seed. 13. III. 12.

Soil treatment	Ammonia and Nitrate per million of dry soil				Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total		16 hrs.	18½ hrs	21 hrs.	23½ hrs.	40 hrs.	64 hrs.	
Untreated	2	24	26	Lower moisture	11	48	172	265	480	534	545
				Higher ,,	28	91	212	308	508	546	560
Heated to 100° C.	11	23	34	Lower moisture	3	35	135	229	486	556	580
				Higher ,,	18	55	157	256	463	526	545
Heated to 58° C.	10	24	34	Lower moisture	20	70	199	306	512	545	564
				Higher ,,	29	100	218	312	487	533	545
Treated with Toluene	8	23	31	Lower moisture	5	41	151	241	474	534	558
				Higher ,,	11	51	165	254	483	543	557
Sand				Lower moisture	13	38	136	209	470	527	537
				Higher ,,	41	119	234	327	487	529	550

Exp. 158, 1.

Same soil. Tomato Seed.

Soil treatment		Seeds germinated after					Total germinating
		40 hrs.	45 hrs.	64 hrs.	72 hrs.	88 hrs.	
Untreated soil.....	Lower moisture	249	303	458	462	468	480
	Higher ,,	228	300	467	474	482	493
Heated to 100° C. ...	Lower moisture	79	106	318	390	441	474
	Higher ,,	36	58	261	324	434	480
,, ,, 55° C. ..	Lower moisture	126	166	400	430	459	483
	Higher ,,	113	147	380	428	465	480
Treated with Toluene	Lower moisture	101	144	361	414	451	479
	Higher ,,	94	149	344	387	445	474
Sand	Lower moisture	18	35	266	364	452	477
	Higher ,,	58	97	332	410	467	488

Exp. 158, 9.

In each case the lower moisture = 31·6 % and the higher moisture = 37·5 %.

TABLE IV (*cont.*).

B. The same soil stored for eight weeks. Turnip seed. 21. V. 12.

Soil treatment	Ammonia and Nitrate per million of dry soil				Seeds germinated after					Total germinating
	Ammonia	Nitrate	Total		16 hrs.	18 hrs.	20 hrs.	23 hrs.	40 hrs.	
Untreated soil	2	27	29	Lower moisture	31	67	128	214	472	548
				Higher ,,	31	80	149	249	495	548
Heated to 100° C.	44	22	66	Lower moisture	39	76	164	268	489	559
				Higher ,,	21	69	134	245	482	559
Heated to 55° C.	41	25	66	Lower moisture	44	92	181	272	425	510
				Higher ,,	48	131	211	311	505	572
Treated with Toluene	45	26	71	Lower moisture	28	75	134	235	433	518
				Higher ,,	36	107	191	302	510	555
Sand				Lower moisture	38	104	167	237	513	572
				Higher ,,	86	169	239	318	480	462

Exp. 158, 36.

In each case the lower moisture = 31·6 % and the higher moisture = 37·5 %.

C. A rich greenhouse soil containing 0·63 % N, 1·93 % $CaCO_3$ and losing 16·9 % on ignition. Turnip seed. 30. IV. 12.

Soil treatment	Ammonia and Nitrate per million of soil				Seeds germinated after						Total germinating
	Ammonia	Nitrate	Total		16 hrs.	18½ hrs.	21½ hrs.	24 hrs.	40 hrs.	64 hrs.	
Untreated soil	13	315	328	Lower moisture		113	302	417	518	542	553
				Higher ,,	34	122	277	390	511	535	545
Heated to 100° C.	64	323	387	Lower moisture		47	241	324	469	503	523
				Higher ,,	12	35	115	240	473	520	551
Heated to 55° C.	—	—	—	Lower moisture		89	246	338	497	525	538
				Higher ,,	27	112	281	381	506	550	568
Treated with Toluene	23	295	318	Lower moisture		70	261	363	520	549	556
				Higher ,,	10	80	172	291	478	513	542
Sand	—	—	—	Lower moisture		52	190	310	516	542	553
				Higher ,,	32	110	265	359	488	528	554

Exp. 158, 27.

TABLE IV (*cont.*).

The same soil. Tomato seed. 1. V. 12.

Soil treatment		Seeds germinated after					Total germinating
		40 hrs.	45 hrs.	48 hrs.	64 hrs.	88 hrs.	
Soil untreated	Lower moisture	59	140	196	407	451	479
	Higher ,,	79	212	269	420	454	479
Heated to 100° C. ...	Lower moisture	2	4	8	66	189	480
	Higher ,,	1	4	4	47	197	508
Heated to 55° C.	Lower moisture	13	27	43	180	320	478
	Higher ,,	17	32	48	222	347	491
Treated with Toluene	Lower moisture	5	20	41	191	341	479
	Higher ,,	4	15	22	190	370	486
Sand	Lower moisture	—	3	12	226	416	479
	Higher ,,	31	72	105	327	433	472

Exp. 158, 31.

In each case the lower moisture = 45 % and the higher moisture = 52 %.

The varying effects of partially sterilised soil on germination are shown in Tables II, III and IV. In a certain number of cases germination is most rapid in the untreated soil, less rapid in the soil heated to 55°, still less in the soil treated with the toluene, and slowest in the soil heated to 100°. The amounts of ammonia in the soil run in the same direction as the extent of the retardation and in these instances it might be supposed that the retardation was approximately proportional to the amount of ammonia present. But there are too many exceptions to allow of this assumption; many cases occur among the poorer soils where germination takes place most rapidly in the soil heated to 55°, and in two or three instances germination is more rapid in the soils heated to 100° or treated with toluene than in the untreated soil. This is with turnip seed; with tomato seed different results are obtained, the order of the soils being practically always as follows: untreated soil (germination most rapid); soil heated to 55°, to 84° or treated with toluene; soil heated to 100° (germination slowest).

The effect of storage. When soils have been stored for some time after partial sterilisation they undergo a change in composition shown by an increase in the total ammonia and nitrates. The increase is most marked in the soil heated to 100° where it takes the form of

ammonia, less marked in the soil treated with toluene where it appears either as ammonia or nitrates, and sometimes still less marked in the soil heated to 55°, but is least in the untreated soil where it occurs only in the nitrates.

The effect on germination is shown in Table III. Of the stored soils those that have been heated to 55° or treated with toluene are generally more favourable or less unfavourable to germination than those heated to 100°, but not invariably. The effect does not pass off with time, but no estimate can be formed as to how much it changes because the untreated soil alters also. There is in fact no unchanging standard whereby measurements could be taken. Further complication arises from the fact that the phenomena depend to some extent on the amount of water present in the soil.

The effect of varying moisture content. As the amount of moisture in the soil is increased so the retarding effect of the soil heated to 100° becomes more marked. This was demonstrated in the following experiment. Tomatoes were sown in a rich soil (a pasture soil) but insufficient water was added for rapid germination: periodical counts showed that only a slight retardation was produced in the soil heated to 100°. On adding more water germination became much more rapid, but the inhibitory effect of the heated soil now manifested itself.

The results were:

Soil treatment	1st Period, insufficient moisture			2nd Period, more water added		Total germinating
	Seeds germinated after			Seeds germinated after		
	64 hrs.	88 hrs.	114 hrs.	136 hrs.	160 hrs.	
Untreated.....	8	18	31	70	113	119
Heated to 100° C.	3	15	31	52	93	115
„ „ 55° C.	8	21	30	65	112	118

21. III. 11. Exp. 115, 45.

More complete evidence is given in Table IV. Soil heated to 55° or treated with toluene generally shows the same phenomena. Similar results were obtained with sand watered with dilute ammonia solution (Table VI), so that the effect does not depend entirely on any property of the soil.

The effect of altered physical conditions. While carrying out the experiments it became evident that some physical change took place on partial sterilisation because the treated and untreated soils showed considerable difference in their behaviour when watered, as already noted by Pickering[1]. Water readily soaked into the untreated soils, but it stood in drops on the others and did not penetrate for some time, especially into the soil heated to 100°. When all the soils received the same weight of water there was an obvious difference in their appearance, the treated soils appearing to be the wettest. This physical difference does not appear to be the determining factor in bringing about the germination phenomena for the following reasons:

1. In all the experiments in Table II there was excess of water.

2. Where less water was used the difference between the untreated and the treated soils did not become more marked but less (Table IV).

3. An aqueous extract of the soil made by stirring up the soil with twice its weight of water and filtering through a Buchner funnel also affected the rate of germination.

The behaviour of the soil extract. Extracts made in the manner stated above were added to quartz sand (40 grams) contained in Petri dishes. Seed was then sown and the experiment was conducted in the usual manner. The results are given in Table V: they show the same kind of differences as were obtained in the experiments with soils, the extract of the soil heated to 55° sometimes accelerating and sometimes retarding germination in comparison with the extract of untreated soil, while the extract of the soil heated to 100° has a retarding effect. In two cases out of the three the soil extracts all retarded germination

TABLE V. *Effect of aqueous extracts of soils on germination.*

A. Extract of greenhouse soil R.C. Turnip Seed. 19. X. 11.

Soil treatment	Seeds germinated after				Total germinating
	16 hrs.	19 hrs.	22½ hrs.	38½ hrs.	
Untreated	84	266	373	500	598
Heated to 100° C.	83	255	381	496	596
,, ,, 55° C.	93	286	382	493	592
Treated with Toluene	78	280	374	494	596

Exp. 135, 22.

[1] This *Journal*, 1910, **3**, 261.

TABLE V (*cont.*).

B[1]. Extract of garden soil as used in Table III, B. Turnip seed. Temp. 19° C. 15. IX. 11.

Soil treatment	Seeds germinated after				Total germinating
	19½ hrs.	21½ hrs.	25 hrs.	37 hrs.	
Untreated	36	124	334	558	568
Heated to 100° C.	18	84	297	576	581
,, ,, 55° C.	26	106	319	561	571
Treated with Toluene	17	84	305	567	580
Distilled water	23	90	290	557	568

Exp. 135, 12.

[1] In this experiment the temperature fell to 14° during the first 12 hours, it was then put up to 23°. A 20 % extract was used in this case.

The data for the soil are given in Table II, B.

C. Extract of pasture soil. Turnip seed. 20. II. 12.

Soil treatment	Seeds germinated after					Total germinating
	17 hrs.	19 hrs.	22 hrs.	24½ hrs.	41 hrs.	
Untreated	29	58	177	306	497	559
Heated to 100° C.	20	49	147	265	473	564
,, ,, 59° C.	24	64	183	308	502	573
Distilled water	36	118	253	366	508	576

Exp. 135, 72.

D. Extract of the same soil. Tomato seed. 7. II. 12.

Soil treatment	Seeds germinated after			Total germinating
	42 hrs.	47 hrs.	66 hrs.	
Untreated	62	223	464	480
Heated to 100° C.	63	224	468	486
,, ,, 55° C.	101	269	468	479
Distilled water	97	263	470	484

Exp. 135, 67.

in comparison with distilled water, in the third case, however, some acceleration was produced by the extracts of untreated soil.

The experiment with the extract of the garden soil (B, Table V) was carried out immediately after that with the soil itself (C, Table III), but no resemblance can be discovered in the results. The conditions, however, are so very different that no strict comparison can be made. Soil behaves entirely differently from sand towards water and dissolved substances. The soil contained 37 per cent. of water and was not very wet, but the sand was absolutely flooded by the same amount so that we had to reduce the percentage to 19. There is no quantitative means of ascertaining when soil and sand are equally moist to seeds or plants, and any attempt to judge of equality in this direction reduces itself to mere guess work. A further difference between soil and sand is that soil has much the greater power of withdrawing dissolved substances from their solutions.

The behaviour of the separate constituents. Free ammonia has a remarkably powerful effect on germination, accelerating it at low concentrations (one or two parts per million), but retarding it at higher concentrations; at some intermediate point it has no effect (Table VI). The effective concentration is, however, influenced considerably by the amount of water present and the temperature. Thus when 19·3 per cent. of water was present four parts per million of ammonia caused a marked acceleration: when 20·6 was present the same amount of ammonia caused no acceleration but a slight initial retardation. A similar result was obtained with soil (Table IV) and we may therefore conclude that it is not due to any soil property. The determining factor in the case of ammonia appears to be the rapidity at which germination takes place; when germination is slow ammonia tends to hasten it, when it is rapid ammonia has less effect but tends to retard it. This is also seen in A and B of Table VI; two parts per million of ammonia considerably hasten germination in the slowly germinating seeds of B, but cease to do so in the more rapidly germinating seeds of A.

In Table VI, D a comparison is instituted between sodium nitrate, ammonium sulphate and free ammonia at equimolar concentrations, 180 parts per million being chosen to correspond with one of the soils under investigation. It will be seen that free ammonia is much the most drastic in its effect, ammonium sulphate comes next and sodium nitrate is the weakest. Further, the mixture corresponding with that in the soil behaves not unlike the soil itself, although, as already stated, no definite comparison can be made.

TABLE VI. *Effect of solutions of ammonia, ammonium sulphate and sodium nitrate on germination.*

A. Ammonia solutions added to pure sand. Turnip seed. 23. V. 11. Moisture = 16·7 %.

Concentration parts of N. per million of moist sand	Seeds germinated in				Total germinating
	13 hrs.	18 hrs.	23 hrs.	61 hrs.	
Nil (water only)...	219	422	495	555	573
1 per million	248	453	495	559	575
2 ,, ...	216	432	496	556	579
10 ,, ...	117	389	483	559	576
100 ,, ...	none	none	none	none	none

Exp. 115, 61.

B. Ammonia solutions added to pure sand. Turnip seed. 26. VI. 11. Moisture = 19·3 %.

Concentration parts of N. per million of moist sand	Seeds germinated in						Total germinating
	15 hrs.	18 hrs.	21 hrs.	24 hrs.	27 hrs.	36 hrs.	
Nil (water only)...	48	224	368	459	551	572	600
1 per million	67	255	394	460	553	575	592
2 ,, ...	80	274	405	465	556	578	600
4 ,, ...	97	278	423	465	551	579	604

Exp. 115, 74.

C. Ammonia solutions added to pure sand. Turnip seed. 3. VII. 11.

Concentration	Seeds germinated in							Total germinating
	16 hrs.	19 hrs.	22 hrs.	25 hrs.	28 hrs.	40 hrs.	64 hrs.	
19·3 % water, no NH_3	38	194	352	423	465	553	586	598
4 per million	73	229	403	464	488	551	584	590
20·6 % water, no NH_3	153	330	430	466	494	539	569	580
4 per million	139	337	427	459	485	539	576	587

Exp. 115, 77.

TABLE VI (*cont.*).

D. Comparison of $(NH_4)_2SO_4$ and $NaNO_3$ made up in concentrations equivalent to those present in a soil. (A 3 in Table III, 9. I. 12.)

The solutions added to pure sand. Turnip seed. 20. I. 12.

Moisture = 20 %.

Concentration parts of N. per million of moist sand	Substance added	Seeds germinated in				Total germinating
		20½ hrs.	23½ hrs.	40 hrs.	64 hrs.	
Nil (water only)	—	217	347	504	539	554
180	$NaNO_3$	125	264	515	569	575
180	$(NH_4)_2SO_4$	8	43	422	523	558
180	NH_4OH	0	0	0	41[1]	165
90 as NH_3	$(NH_4)_2SO_4$	42	139	438	499	518
83 as nitrate	$NaNO_3$	—	—	—	—	—

Exp. 2, 61.

[1] By this time some of the NH_3 had volatilised.

E. Sand. $NaNO_3$ dilute solutions. Tomato seed.

Concentration	Seeds germinated after	
	40 hrs.	45 hrs.
14 parts N. per million	191	348
7 ,, ,,	222	365
1·4 ,, ,,	270	363
Distilled water	251	364

16. II. 12. Exp. 135, 70.

Tomato seed proved highly susceptible to free ammonia but was much less affected by solutions of sodium nitrate; it showed signs of being stimulated in germination by dilute solutions but retarded by stronger ones (E, Table VI).

The subsequent growth of plants in partially sterilised soils.

1. *The seedling and young plant.*

The general nature of the effects observed during the seedling and early stages have already been described. It now remains to give some of the details of the experiments.

a. Soils heated to 100° C. *Poor soils.* In poor soils there is almost invariably an acceleration in growth right from the outset. The food supply being the factor that limits plant growth, the increase in food supply consequent on partial sterilisation is accompanied by an increase

in growth. But another factor also comes into play. These poor soils are deficient in organic matter and therefore tend to be rather sticky, but after they have been heated their mechanical condition is often much improved. The young seedling therefore has a better chance of pushing ahead. On Feb. 24th tomato seeds were sown in poor arable soil ("Little Hoos," see Table II, A for composition); out of twenty sown in each set the numbers coming up were:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.	Soil treated with Toluene	Soil treated with CS_2
March 10	1	5	1	2	1
,, 11	3	8	6	4	4
,, 13	7	11	8	9	5
,, 14	7	14	8	9	5
and the spread of the cotyledons from tip to tip was:					
,, 13	1	7	2	3	1 cms.
,, 14	2	11	4	4	1 ,,

In a warmer house the soils heated to 100° did not stand out so remarkably, but were no better than the soils treated with the toluene and carbon disulphide.

Richer soils. In richer soils a very different effect is produced. The seedlings are distinctly retarded in coming up. On March 6th 100 tomato seeds were sown in a glass house soil containing 0·33 per cent. nitrogen and losing 7·9 per cent. on ignition; the following numbers of seedlings appeared on the given dates:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.
March 25	10	0	5
,, 26	14	1	12
,, 27	18	3	17
,, 28	24	6	21

and so on right through to the time when all were up.

This appears to be a new retardation additional to that affecting germination, for it continues to operate over many days, and is not confined to the germination period.

Poor seed suffers considerably in the soil heated to 100° and much of it does not yield plants at all. This causes a further marked

difference between the untreated and the steamed soils. Good seed, however, gives rise to the same number of plants in both cases.

Later on the roots begin to grow more quickly in the heated soil, even while the shoots are still retarded. This may happen during the cotyledon stage if the retardation has only been slight:

Soil *MT*, containing 0·26 °/₀ N, 1·0 °/₀ $CaCO_3$ and losing 7·8 °/₀ on ignition.
25 tomato seeds sown Dec. 5th.

	Untreated soil	Soil heated to 98° C.	Soil heated to 55° C.	Soil treated with Toluene	Soil treated with carbon disulphide
Number up on Dec. 19	4	2	8	4	6
,, ,, 20	4	4	10	6	6
,, ,, Jan. 9	5	8	15	10	10
Spread of cotyledons on Jan. 18	3·6	3·3	4 1	2·5	2·5 cms.
Root length on Jan. 18	0·9	2·7	2·7	3·8	2·6 ,,

or it may be later on when the fourth leaves are out that quicker growth starts. Retardation at this later stage is shown in Fig. 5. Plants lifted at this early stage were found to have the following average weights per plant:

	Untreated soil	Soil heated to 98° C.	Soil heated to 55° C.	Soil treated with Toluene	Soil treated with carbon disulphide
Shoot, green weight grms.	0·65	0·31	1·33	1·27	1·06
,, dry ,, ,,	0·07	0·03	0·13	0·115	0·085
Root, fresh ,, ,,	0·08	0·03	0·20	0·18	0·10
,, dry ,, ,,	0·007	0·004	0·020	0·018	0·009

b. Soils heated to 55° C. or treated with toluene and carbon disulphide. These generally cause an acceleration (especially the soil heated to 55°) but sometimes a retardation. Tomatoes sown on Feb. 24th in a greenhouse compost containing 0·35 °/₀ N., 0·11 °/₀ $CaCO_3$ and losing 9·0 °/₀ on ignition came up as follows:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.	Soil treated with Toluene
March 8	3	0	2	4
,, 9	6	8	5	10
,, 10	9	9	9	13
,, 11	12	10	15	15
,, 13	15	15	17	17
,, 14	17	16	17	18

20 seeds were sown in each set.

The spread of the cotyledons in cms. was:

	Untreated soil	Soil heated to 100° C.	Soil heated to 55° C.	Soil treated with Toluene
March 9	3	0	1	4
,, 10	5	4	3	8
,, 11	9	9	9	13
,, 13	13	11	14	17
,, 14	15	13	17	17

The experiment was made in duplicate in another house at rather higher temperature but with entirely different results, the plants on the treated soils being all behind those on the untreated soil. Subsequent repetition in the same house gave a result similar to the one set out above, treatment of the soil with toluene and carbon disulphide causing a slight acceleration while heat at 100° C. caused a marked retardation. This variation in the results is typical, and illustrates the close relationship between acceleration and retardation in these early stages of plant growth.

2. *The adult plant.*

The times of flowering and ripening. Tomatoes, cucumbers and chrysanthemums growing on the partially sterilised soil flowered several days before those on the untreated soil, and the fruit of the first two was not only earlier but more prolific. This fact is remarkable because as a general rule excess of nitrogenous food (such as may be supposed to result from partial sterilisation) retards flowering and ripening; the tomato grower greatly objects to rank growth as being detrimental to fruit production. Fig. 1 (p. 254) shows the total weight of fruit obtained from tomato plants grown on untreated and partially sterilised soils, the manuring and all other conditions being uniform for the whole series. The plants were grown in pots and stopped at four trusses. Details of the experiment are recorded in the *Journal of the Board of Agriculture*, 1913, **19**, 809, and need not be repeated here.

Fig. 7 shows blooms of chrysanthemums grown under similar conditions; the untreated soil is seen to give poorer growth and later blooms than the treated soils excepting only the steamed soil. With other varieties the plants on the steamed soil were ahead of those on the untreated soil; the blooms were also larger and brighter.

To some extent our result is due to the conditions of the experiment; the bad effect of rankness is much less evident when the plants are

grown in pots than when they have the less restricted root range of a border.

Composition of the dry matter. Various tables of analyses are given in earlier publications showing the composition of the dry matter of crops grown under similar conditions on partially sterilised and on untreated soils[1]. The percentage of nitrogen in the dry matter is in almost all cases found to be higher on crops grown on partially sterilised soils as also is the percentage of phosphoric acid where the soil has in the past been well dunged (*e.g.* the hop garden soil in the 1907 paper).

In further confirmation of these results the following may be given:

Percentage of nitrogen in dry matter of barley, 1910 crop.

Soil untreated	2·83	Soil untreated	2·98	Soil untreated	3·43
,, treated with Toluene	3·44	,, heated to 45° C.	3·31	,, dried at 40° C. for 24 hrs.	3·39
CaS	3·32	,, ,, 55° C.	3·61	,, ,, ,, 5 days	3·56
Shell petrol	3·39	,, ,, 65° C.	3·67	,, ,, ,, 10 ,,	3·62
Ether	3·33	,, ,, 75° C.	3·55		
		,, ,, 85° C.	3·43		
		,, ,, 100° C.	3·58		

Subsequent crops on the same soil gave similar results. The exception to the general rule occurs when the plants on the partially sterilised soils have made conspicuously more growth than those on the untreated soil. For example tomato plants pulled up just before flowering time after a period of markedly accelerated growth on the partially sterilised soils gave the following results:

Soil treatment	Untreated	Heated to 98° C.	Treated with Formaldehyde	Treated with Pyridene	CaS	Petrol	Toluene	Phenol
Dry matter grams	7·1	26·8	16·6	13·2	13·2	12·1	11·8	9·0
N. % in dry matter	2·92	2·52	2·22	3·17	2·67	2·46	2·50	2·43

Analyses have been made of tomato plants left to grow till fruiting had finished so as to ascertain how far the nitrogen, phosphorus and potassium had been exhausted from the roots and stems, etc. and transferred to the fruit. The process was found to take place more completely on partially sterilised than on untreated soils. The fruit of plants raised on partially sterilised soil contained in most cases a higher percentage of nitrogen, and if the soil had previously been

[1] This *Journal*, 1907, **2**, 313 *et seq.*; 1909, **3**, 122.

TABLE VII. *Composition of dry matter of tomato plants grown in untreated and in partially sterilised soils.*

1. Amounts of Nitrogen, per cent. of dry matter and weight in grams per plant.

Pasture Soil *TF* containing 0·59 % N, 0·15 % $CaCO_3$ and losing 13·5 % on ignition. This soil had not received dung.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	4·94	19·15	4·70	2·05	1·13	2·91	0·101	0·216	0·137	28·79	0·45
Heated to 98° C.	2·77	25·45	18·56	1·58	1·47	3·04	0·044	0·373	0·565	46·78	0·98
,, +Basic slag	1·73	22·89	19·45	1·91	1·55	3·11	0·033	0·355	0·606	44·07	0·99
,, 55° C.	2·99	22·66	17·68	1·61	1·16	2·06	0·048	0·261	0·364	43·33	0·67
Treated with Toluene	2·57	21·69	18·94	1·81	1·08	3·01	0·047	0·234	0·569	43·20	0·85
,, ,, CS_2	2·34	22·15	16·81	1·58	1·17	3·06	0·037	0·259	0·514	41·30	0·81

Soil *MC*. Old tomato-house soil.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	5·14	16·23	9·03	2·30	1·01	1·72	0·118	0·163	0·156	30·39	0·44
Heated to 98° C.	6·84	31·25	24·40	1·57	0·82	2·12	0·107	0·255	0·516	62·49	0·87
,, +Basic slag	9·97	29·52	28·40	1·39	0·86	1·94	0·138	0·254	0·551	67·89	0·94
,, 45° C.	4·06	18·31	14·88	2·36	1·13	1·83	0·096	0·206	0·272	37·24	0·57
Treated with Toluene	4·05	23·76	16·74	2·35	1·04	2·00	0·095	0·248	0·334	44·55	0·68
,, ,, CS_2	3·95	22·78	17·56	2·41	1·01	1·73	0·095	0·229	0·304	44·29	0·63

TABLE VII.—*continued.*

Soil *SC*. Old tomato-house soil containing 0·33 % Nitrogen and losing 7·9% on ignition.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	3·67	22·77	31·87	2·00	0·91	1·74	0·073	0·207	0·555	58·31	0·83
Heated to 98° C.	5·85	33·86	40·28	1·74	1·14	2·05	0·102	0·386	0·825	80·00	1·31
,, 55° C.	3·27	26·02	28·11	1·83	1·04	2·00	0·060	0·270	0·561	57·40	0·89

Soil *MT*. Old tomato-house soil containing 0·26 % N, 0·97 % $CaCO_3$ and losing 6·0 % on ignition.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	9·53	32·12	25·23	2·24	0·91	1·75	0·213	0·292	0·440	66·88	0·95
Heated to 98° C.	8·73	55·58	39·19	1·67	0·90	2·13	0·146	0·502	0·836	103·50	1·48
,, 55° C.	5·03	33·52	26·23	1·93	0·81	1·89	0·097	0·274	0·495	64·78	0·86
Treated with Toluene	8·10	35·50	33·66	2·31	0·78	2·15	0·187	0·276	0·723	77·26	1·19
,, ,, CS_2	8·37	33·99	34·29	2·31	0·83	2·26	0·194	0·281	0·776	76·65	1·25

Soil *WW*. Old cucumber-house soil containing 0·72 % N, 0·92 % $CaCO_3$ and losing 19·9 % on ignition.

	Weight per plant of dry matter in			Nitrogen per cent. in dry matter of			Weight of Nitrogen in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	Nitrogen
Untreated	2·10	23·67	13·87	1·99	1·25	2·22	0·042	0·296	0·307	39·64	0·65
Heated to 98° C.	8·53	38·64	25·77	1·60	1·17	2·22	0·136	0·453	0·571	72·94	1·16
,, +Basic slag	8·28	39·87	26·60	1·57	1·24	2·65	0·130	0·494	0·704	74·75	1·33
,, 55° C.	2·03	29·13	11·70	2·09	1·10	2·08	0·042	0·320	0·243	42·86	0·61
Treated with Toluene	2·49	32·87	13·85	2·17	1·06	2·18	0·054	0·348	0·301	49·21	0·70
,, ,, CS_2	1·99	26·74	15·75	2·29	1·24	2·42	0·045	0·332	0·381	44·48	0·76

2. Amounts of phosphorus (expressed as P_2O_5), per cent. of dry matter and weight per plant. Estimated as P_2O_5 in ash. Soil *TF* containing 0·16 % P_2O_5 soluble in boiling conc. HCl and 0·016 % soluble in 1 % Citric acid.

Per cent. of N, etc. given above.

	Weight per plant of dry matter in			P_2O_5 per cent. in dry matter of			Weight of P_2O_5 in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	P_2O_5
Untreated	4·94	19·15	4·70	0·560	0·590	1·458	0·028	0·113	0·069	28·79	0·21
Heated to 98° C.	2·77	25·45	18·56	0·340	0·257	—	0·009	0·065	—	46·78	—
,, +Basic slag	1·73	22·89	19·45	0·311	0·299	0·986	0·005	0·068	0·192	44·07	0·27
,, 55° C.	2·99	22·66	17·68	0·356	0·361	0·853	0·011	0·082	0·151	43·33	0·24
Treated with Toluene	2·57	21·69	18·94	0·565	0·186	1·103	0·015	0·040	0·209	43·20	0·26
,, ,, CS_2	2·34	22·15	16·81	0·333	0·319	1·379	0·008	0·071	0·232	41·30	0·31

TABLE VII.—*continued.*

Soil *MT* containing 0·39 % P_2O_5 soluble in boiling conc. HCl and 0·23 % soluble in 1 % Citric acid.

	Weight per plant of dry matter in			P_2O_5 per cent. in dry matter of			Weight of P_2O_5 in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	P_2O_5
Untreated	9·53	32·12	25·23	0·623	0·375	0·854	0·059	0·120	0·215	66·88	0·39
Heated to 98° C.	8·73	55·58	39·19	0·564	0·547	1·084	0·049	0·304	0·425	103·50	0·78
,, 55° C.	5·03	33·52	26·23	0·596	0·400	1·070	0·030	0·134	0·281	64·78	0·44
Treated with Toluene	8·10	35·50	33·65	0·699	0·291	0·967	0·057	0·103	0·325	77·26	0·49
,, ,, CS_2	8·37	33·99	34·29	0·610	0·348	1·020	0·051	0·118	0·350	76·65	0·52

3. Amounts of potassium (expressed as K_2O), per cent. of dry matter and weight per plant.

Soil *TF* containing 0·39 % K_2O soluble in boiling conc. HCl and 0·08 % soluble in 1 % Citric acid.

	Weight per plant of dry matter in			K_2O per cent. in dry matter of			Weight of K_2O in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	K_2O
Untreated	4·94	19·15	4·70	1·720	2·519	6·149	0·085	0·482	0·289	28·79	0·86
Heated to 98° C.	2·77	25·45	18·56	0·919	1·318	—	0·025	0·335	—	46·78	—
,, +Basic slag	1·73	22·89	19·45	0·567	1·626	5·487	0·010	0·372	1·067	44·07	1·45
,, 55° C.	2·99	22·66	17·68	0·824	1·568	4·971	0·025	0·355	0·879	43·33	1·26
Treated with toluene	2·57	21·69	18·94	1·287	1·309	5·741	0·033	0·284	1·087	43·20	1·40
,, ,, CS_2	2·34	22·15	16·81	0·839	1·688	6·910	0·020	0·374	1·162	41·30	1·56

Soil *MT* containing 0·44 % K_2O soluble in boiling conc. HCl and 0·07 % soluble in 1 % Citric acid.

	Weight per plant of dry matter in			K_2O per cent. in dry matter of			Weight of K_2O in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	K_2O
Untreated	9·53	32·12	25·23	1·806	3·282	5·124	0·172	1·054	1·293	66·88	2·52
Heated to 98° C.	8·73	55·58	39·19	1·789	3·762	5·736	0·156	2·091	2·248	103·50	4·49
,, 55° C.	5·03	33·52	26·23	2·064	2·864	5·930	0·104	0·960	1·555	64·78	2·62
Treated with Toluene	8·10	35·50	33·66	2·070	2·971	6·529	0·168	1·054	2·198	77·26	3·42
,, ,, CS_2	8·37	33·99	34·29	2·201	3·320	6·528	0·184	1·128	2·238	76·65	3·55

4. Amounts of Ca (expressed as CaO), per cent. of dry matter and weight per plant.

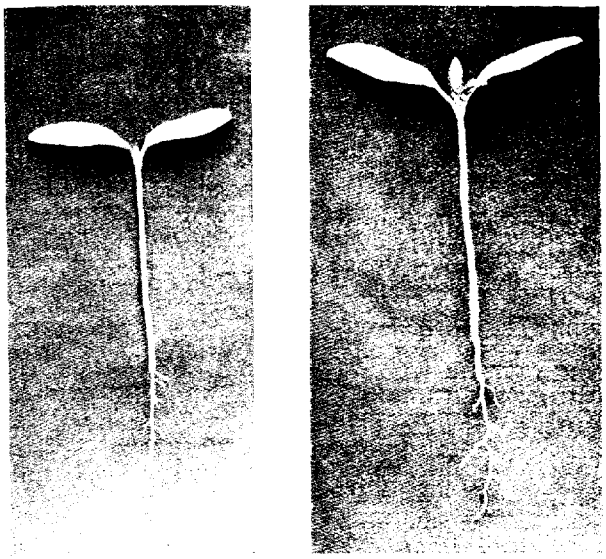
Soil *TF*.

	Weight per plant of dry matter in			CaO per cent. in dry matter of			Weight of CaO in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	CaO
Untreated	4·94	19·15	4·70	0·205	6·521	0·104	0·010	1·249	0·005	28·79	1·26
Heated to 98° C.	2·77	25·45	18·56	0·343	8·612	—	0·010	2·192	—	46·78	—
,, +Basic slag	1·73	22·89	19·45	0·297	8·222	0·076	0·005	1·882	0·015	44·07	1·90
,, 55° C.	2·99	22·66	17·68	0·347	8·688	0·099	0·010	1·969	0·018	43·33	2·00
Treated with Toluene	2·57	21·69	18·94	0·485	8·044	0·111	0·012	1·745	0·021	43·20	1·78
,, ,, CS_2	2·34	22·15	16·81	0·303	7·176	0·086	0·007	1·589	0·014	41·30	1·61

TABLE VII.—*continued.*

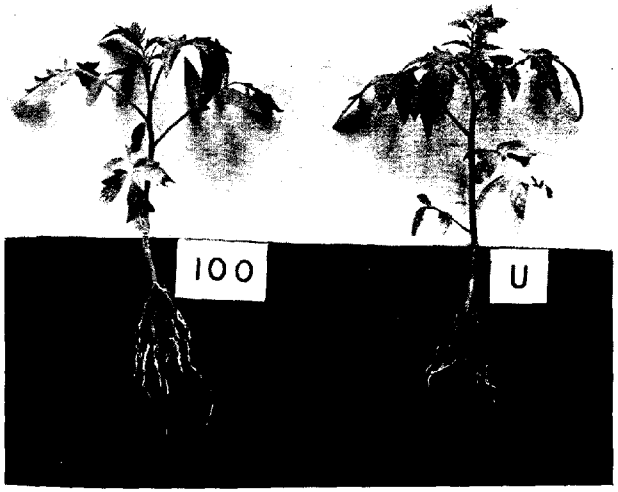
Soil *MT.*

	Weight per plant of dry matter in			CaO per cent. in dry matter of			Weight of CaO in			Total weight of	
	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Root	Stem and leaf	Fruit	Dry matter	CaO
Untreated	9·53	32·12	25·23	0·242	7·074	0·065	0·023	2·272	0·016	66·88	2·31
Heated to 98° C.	8·73	55·58	39·19	0·374	6·346	0·026	0·033	3·527	0·010	103·50	3·57
,, 55° C.	5·03	33·52	26·23	0·275	4·461	0·047	0·014	1·495	0·012	64·78	1·52
Treated with Toluene	8·10	35·50	33·66	0·231	6·908	0·038	0·018	2·452	0·013	77·26	2·48
,, ,, CS_2	8·37	33·99	34·29	0·298	5·797	0·051	0·025	1·971	0·017	76·65	2·01



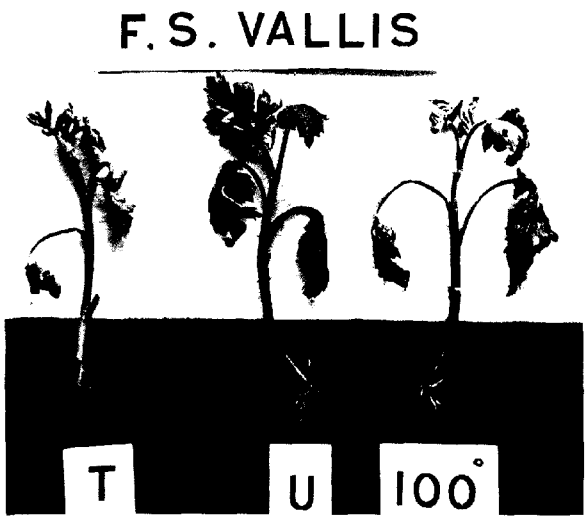
Soil heated to 100° C. Untreated soil.

Fig. 2. Retardation in early stages of growth in soil heated to 100° C. Tomato seedlings.



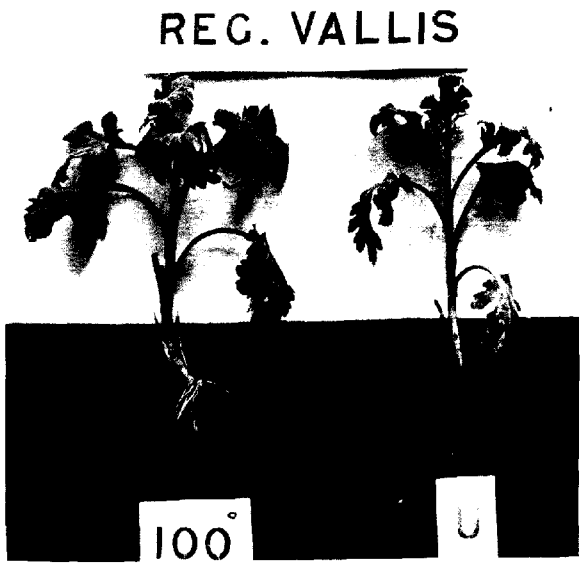
Soil heated to 100° C. Untreated soil.

Fig. 3. Accelerated root development in later stages in soil heated to 100° C. Tomato seedlings.



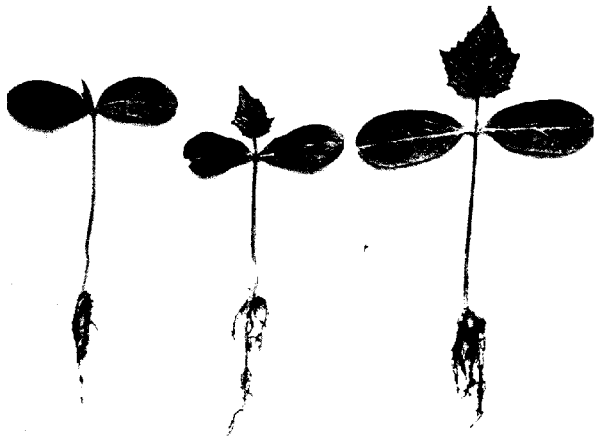
Soil treated with toluene. Untreated soil. Soil heated to 100° C.

Fig. 4*a*. Retardation in root formation in treated soils. Chrysanthemum cuttings, var. F. S. Vallis.



Soil heated to 100° C. Untreated soil.

Fig. 4*b*. Accelerated root development in later stages in soil heated to 100° C. Chrysanthemum cuttings, var. Reginald Vallis.

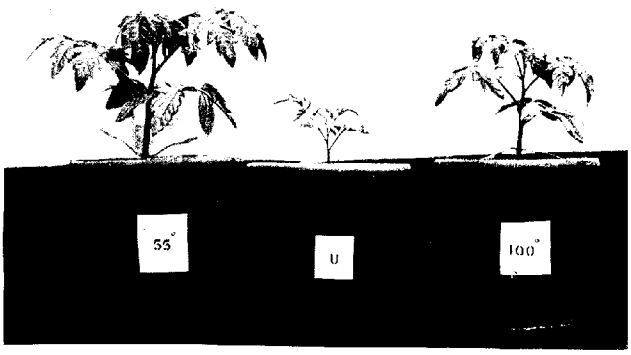


Soil heated to 100° C. Untreated soil. Soil heated to 55° C.

Fig. 5 *a*. Acceleration in early stages of growth in soil heated to 55° C., and retardation in soil heated to 100° C. Cucumber seedlings.

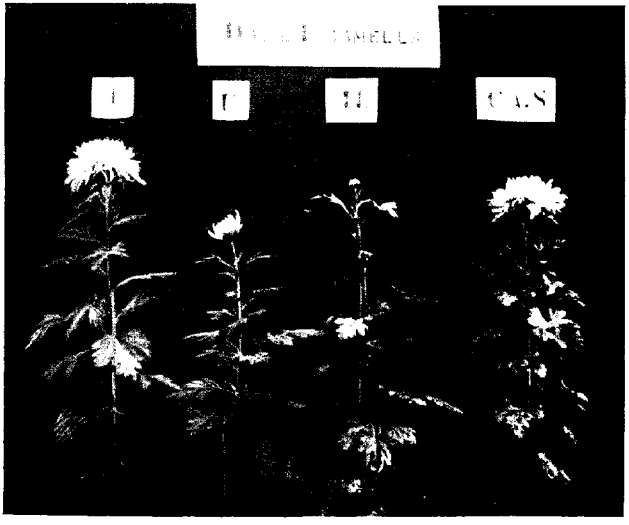
Soil heated to 50° C. Untreated soil. Soil heated to 100° C.

Fig. 5 *b*. Acceleration in early stages of growth in soil heated to 50° C., and retardation in soil heated to 100° C. Tomato seedlings.



Soil heated to 55° C. Untreated soil. Soil heated to 100° C.

Fig. 6. Remarkable acceleration sometimes produced later on in soil heated to 55° C. Tomato plants.



Soil treated with toluene. Untreated soil. Soil heated to 100° C. Soil treated with calcium sulphide.

Fig. 7. Earlier flowering on some of the partially sterilised soils. Chrysanthemums, var. David Ingamells.

dunged, a higher percentage of phosphoric acid and of potassium than that of plants raised on untreated soil; it also had a sweeter taste. The roots of plants grown on soil heated to 100° contained less nitrogen, phosphoric acid and potash than those of plants grown on untreated soil. The roots of plants grown on soil heated to 55°, or treated with antiseptics, do not always show this relationship nor do the stems and leaves.

SILVER-LEAF DISEASE (II).

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Introduction.

The present paper is an account of observations and experiments in connexion with Silver-leaf Disease which have been made since the publication of my paper on the same subject in the *Journal of Agricultural Science* for 1911. I am indebted to the Board of Agriculture for giving a grant to assist this work and to the Cambridge University School of Agriculture for placing at my disposal a plot of land on its farm for most of the fruit trees used in the experiments. I have also to thank Mr R. I. Lynch, Curator of the Botanic Gardens, Cambridge, for providing accommodation for other trees.

In a previous paper[3] on this disease further evidence was given of the ability of the fungus, *Stereum purpureum*, to induce the phenomenon of Silver-leaf and upon it the chief responsibility was laid for the losses caused by the malady in the fruit-growing districts of this country. In that paper (p. 141) I pointed out it would be rash to say that *Stereum purpureum* was the *only* cause of Silver-leaf. Recent investigations have strengthened this view, for specimens of silvered foliage have been seen which I am unable to attribute to the action of *Stereum purpureum*.

Subsequent remarks may be anticipated by saying that the silvering of foliage is a widespread phenomenon which is probably induced by various means, the chief one of which in the fruit-growing districts of this country being the fungus *Stereum purpureum*.

Since my paper was published in 1911, Mr H. T. Güssow[5] has written an account of the disease as it occurs in Canada. He also emphasises the part which *Stereum purpureum* plays in causing Silver-leaf disease in fruit trees, and in general his observations are in close agreement with my own.

All the silvered leaves I have examined exhibit the following features:—The upper epidermis is more or less loose from the palisade cells. There is a marked tendency for the mesophyll cells to fall asunder when sections of the leaf are cut, and so pronounced is this, that it is sometimes impossible to obtain sections of silvered leaves which will hold together. Cavities in walls of epidermal cells as described by Percival[3] are sometimes present but I agree with Güssow that they are not invariably found in silvered leaves. The phenomenon of silvering is primarily due to the accumulation of air either below the epidermal cells or in the cavities of their walls, the presence of air in these places interfering with the normal reflection of light from the surface of the leaf.

Field Observations.

The following supplementary account of the distribution of Silver-leaf should be read in connection with my earlier paper. As indicated there, it has been an almost constant experience to find fructifications of *Stereum purpureum* growing on dead branches of Plum trees which bear silvered foliage on other, living, branches. A photograph of such a tree is shewn in Fig. 1, Plate XII. As the death of such trees proceeds, *Stereum purpureum* develops in increasing abundance both on the branches and on the trunk. On one occasion the fructifications of *Polyporus adustus* as well as of *Stereum purpureum* were found to be growing from the trunk of a silvered Plum tree and *Fomes igniarius* has been seen growing in company with *Stereum purpureum* on several silvered Plum trees, but there is as yet no reason for thinking that either *Polyporus adustus* or *Fomes igniarius* is a cause of Silver-leaf. The latter fungus is frequently found on the branches and trunks of Plum trees which bear normal foliage.

In addition to "Victorias" and "Czars," trees of the following varieties of Plums have been seen to be affected with Silver-leaf: Early Rivers, Pond's Seedling, Monarch, Gisborne, Transparent Gage, Purple Gage, and Green Gage, but these varieties are much less susceptible to the disease than are "Victorias" and "Czars." A large number of branches of silvered Plum trees belonging to various varieties and growing in many different fruit gardens have been examined in order to ascertain whether discoloured wood was constantly present in them or in other portions of the tree with which these branches were directly connected. Such discoloured wood has been

found to be invariably associated with the silvered foliage of Plum trees gathered from plantations and this discolouration (cf. Plate XIII, Fig. 4) is strictly comparable with that found in branches artificially inoculated with *Stereum purpureum*. Stained longitudinal sections of such wood shew abundant hyphae in the water-conducting elements which are often partly filled with a gummy substance to which the discolouration is primarily due. The diseased wood is frequently found a considerable distance below the silvered foliage. The amount of discoloured wood as seen in transverse sections varies considerably, a sector of less than 90° being sometimes present, while at other times an area of more than 180° is dark brown in colour. In these cases the zone of discoloured wood sometimes extends from pith to cambium but more often, as in Fig. 4, a narrow band of healthy wood remains in contact with the cambium. Occasionally several discoloured zones are scattered amongst the healthy tissues. Longitudinally the discoloured wood extends a considerable distance and it can frequently be traced back to the larger branches and to the trunk of the tree. The bark of a diseased branch is not affected as soon as the wood, but once it is attacked, the fungus advances in it, as in the wood, more rapidly in a longitudinal than in a lateral direction. Trunks of Plum trees which have been cut down in an advanced stage of disease always contain a large amount of discoloured wood. Fructifications of *Stereum purpureum* frequently develop on the dead bark of one side of a branch or trunk even though the opposite side is still sound.

The distribution of silvered trees in a plantation of Plum trees is usually sporadic unless the plantation is badly neglected, but in a number of cases in which Plum trees have been cut back for various reasons Silver-leaf has developed on almost every tree subjected to this operation. In one such case the larger branches of twenty healthy Plum trees about 20 years of age were cut back during the autumn of 1910 so that only 2 or 3 feet of each of these branches remained. Branches put forth during 1911 were re-grafted in the spring of 1912. None of these grafts grew and when the trees were examined in May 1912 side shoots arising from the branches and trunk and also suckers springing from the root stock were seen to be silvered. Each one of the twenty trees was affected in the same manner. During October, fructifications of *Stereum purpureum* developed in enormous numbers on each of these trees, both on the branches and on the trunk. The large surfaces exposed when the trees were cut back had not been protected in any way and one can readily imagine the facility given to

Stereum purpureum to effect an entrance by this neglect. The fact that fructifications developed at the base of the trunks of certain trees during October 1912 shewed that the fungus had made rapid progress in the tissues.

During the early part of 1912, Mr W. O. Backhouse, late of the John Innes Horticultural Institution, and Mr M. A. Bailey of the same Institution informed me that some seedling Plums which were growing there exhibited the phenomenon of Silver-leaf. Mr Backhouse gave me the following particulars about these seedlings. The seeds were germinated in 2 inches of soil in boxes and the seedlings became silvery at about the time the roots became cramped. When the seedlings were potted they began to recover their normal appearance. The percentage of seedlings which became silvered before being potted varied in different varieties. Thus "Victorias" were affected to an extent of about 50 per cent., "Magnum Bonums" to about 20 per cent., and "Pershores" also to some extent. It is noteworthy that "Czars" grown under the same conditions were entirely unaffected although this variety in fruit plantations is frequently attacked by Silver-leaf disease. The seeds from which these seedlings were derived were obtained from healthy trees. The "Victoria" seedlings which came from "selfed" flowers were more affected with Silver-leaf than those derived from flowers "naturally" pollinated. Some of these seedlings were sent to me and I examined them with the following results: The silvering was strictly comparable with that which occurs in adult trees. The epidermis was partly free from the underlying palisade tissue and on trying to cut sections of the leaf there was a decided tendency for the mesophyll cells to fall asunder one from the other. There was no evidence of fungus attack in either leaf, stem, or root. As already stated, the seedlings began to recover when given more room in which to grow and upon examining them in August, I saw that the recovery of the foliage to its normal appearance was well advanced. I came to the conclusion that in such a case as this the phenomenon of Silver-leaf was not caused by *Stereum purpureum*. Güssow[5] mentions some cases of the appearance of Silver-leaf in seedling plums, and suggests the possibility either that the disease may be perpetuated by means of the seed or that the seedlings may become infected at a very early stage.

For two years in succession Silver-leaf has been reported on Sloe trees (*Prunus spinosa*) in the Isle of Wight. By the kindness of Miss E. Dale I have been able to examine foliage of one of these trees and

the silvering has been found to be strictly comparable with that occurring in cultivated Plum trees.

Although I have seen comparatively few adult Apple trees shewing Silver-leaf I have frequently found scions of regrafted trees that are silvered.

One group of regrafted Apple trees ("Blenheim Orange") which I saw last summer may be described. Fifteen of these trees were cut back three years ago and were regrafted with scions of either the "Grenadier" or "Jubilee" variety. The grafts grew until the spring of 1912 when those on twelve of the trees rapidly died. When these trees were examined in August, 1912, each stock shewed innumerable sporophores of *Stereum purpureum* growing from the bark, and there can be little doubt that this fungus killed the trees (Plate XIII, Fig. 5). The top of one of these trees was sawn off and examined in detail in the laboratory. When split longitudinally the upper part of the stock was seen to be almost completely discoloured, but there was more healthy wood in one of the grafts, thus shewing that it was the stock which had been most severely affected. Hyphae were present in abundance in sections of the discoloured wood. Some portions of the bark of the stock were still healthy and on these no fructifications were found. Grafts of the other three trees were living and were silvered. In longitudinal section the upper part of the stock shewed much less discoloured wood than in the case previously described and the graft was quite sound. Hyphae were found in sections of the discoloured wood of the stock. Such a tree was probably attacked by *Stereum purpureum* but less severely than the trees bearing dead grafts.

Another example of failure of regrafted Apples may be described. In the spring of 1911 four Apple trees ("Cox's Orange Pippin") were regrafted. None of the grafts "took" and by the autumn of the same year *Stereum purpureum* was growing in great abundance on the exposed surfaces of the stock. One of these trees was subsequently cut back to within 9 inches of the ground and the exposed surface covered with clay. At a later date fructifications of *Stereum purpureum* developed even over the clay.

As already mentioned in my previous paper, silvering of Apple grafts is of frequent occurrence even when the scions grow vigorously. All the leaves of such grafts or only a few of them may become silvered. This happens frequently in Cambridgeshire when scions of "Bramley Seedling" are used, but in a large number of these cases the shoots grow out of the malady in the course of time. In one plantation

21 out of 29 Apple trees regrafted with scions of "Bramley Seedling" shewed silvering of the leaves during 1912. It is doubtful whether all such examples of Silver-leaf are due to *Stereum purpureum* for in some which have been examined the amount of discoloured wood in the upper part of the stock is small, the diseased tissues extending only an inch below the exposed surface three years after the stock was cut back. This amount of discolouration is no greater than frequently occurs during the processes of decay that normally affect the exposed extremities of trees which are not protected from the action of micro-organisms.

In a recent conversation Professor Barker of the National Fruit and Cider Institute told me he has seen Apple shoots which have been cut from healthy trees and kept in the air for a short time assume a silvery appearance. This affection which concerned "King of the Pippins" and other varieties of Apples appeared only during the autumn and was irregular in occurrence. I hope to have an opportunity of examining this phenomenon another year. Whether it is Silver-leaf or some other malady allied to it, the affection is evidently not due to the action of *Stereum purpureum*.

While writing of the genus Pyrus I may mention that a branch of a tree of *Pyrus prunifolia* (the Siberian Crab) growing at the Botanic Gardens, Cambridge, was silvered during the summer of 1912.

Several other silvered Gooseberry bushes and Red Currant bushes have been seen since the publication of my previous account; *Stereum purpureum* was growing on dead wood at the base of some of the Red Currant bushes.

A silvered Laburnum tree has been under observation during 1912. At the end of August last, fructifications of *Stereum purpureum* developed in abundance on a dead lateral branch. This tree had been severely pruned in previous years and the wounds thus made doubtless offered facility for the entrance of the fungus.

In February last I saw some plants of the White Dead Nettle (*Lamium album*) the leaves of which were silvered. This affection appeared to be the same in character as Silver-leaf in fruit trees. The upper epidermis was puckered and somewhat shrivelled in places and it could be readily torn away from the underlying mesophyll. It was difficult to obtain section of the silvered leaves on account of the tendency for the mesophyll cells to fall asunder. Other White Dead Nettle plants observed towards the end of March were seen to be silvered, but in this case only the older leaves of the shoots exhibited the phenomenon of silvering. No sign of fungus attack was visible in

these Dead Nettle plants, and the conclusion drawn was that in this case also Silver-leaf could not be attributed to *Stereum purpureum*.

THE HABITATS AND CHARACTERS OF *STEREUM PURPUREUM*.

Considerable attention has been paid to noting the various kinds of plants on which *Stereum purpureum* fructifies in nature. Sporophores of this fungus have been found in England on dead tissues (wood and bark) of the following trees: Plum, Apple, Red Currant, Willow (various species), Poplar (various species), Birch, Beech, Sycamore, and Laburnum, and doubtless this list will be extended with fuller observation. As is the case with many other fungi which also live a similar, partly saprophytic, partly parasitic, existence, fructifications of *Stereum purpureum* are developed only on dead tissues whether the fungus has been living recently as a saprophyte or as a parasite. As will be described later, pieces of sporophore obtained from a dead stump of a Birch tree in the midst of a wood readily induced Silver-leaf in Plum trees.

The closely allied fungus *Stereum hirsutum* is found commonly on dead Oak and Hazel but I have only once seen it on a member of the Plum family. In this case it was growing on a dead part of the trunk of a Damson, the foliage of which was healthy.

As is well known, the fructifications of *Stereum purpureum* are extremely variable in form (Plates XII and XIII, Figs. 1, 2, 3 and 5). Sometimes they are resupinate, and at other times profusely imbricate. On a branch of a Plum tree which is directly horizontally the fructifications are usually confined to the under surface, from which they project only a short distance. When the sporophores develop on the trunk or on a branch which is directed upwards they are usually densely imbricate. When young the sporophores are purplish in colour but they become dingy with age. The sterile surface is hairy when free from the substratum. The fructifications of *Stereum purpureum* may be found at all times of the year. They develop from the vegetative mycelium only after a spell of wet weather and appear in particular abundance after heavy rains during the autumn. The capacity of the sporophores to dry up and spore again under moist conditions has been referred to in the previous paper. According to my experience the spores are $5—7\mu \times 3—4\mu$ in size, these dimensions being rather smaller than those given by Massee[6].

A method of growing the fungus from spores on blocks of Plum wood has already been described[2, 3]. The sporophores referred to there

have constantly lacked the consistency and form of sporophores as they occur in nature. Nevertheless basidia and spores have been frequently observed on these rudimentary sporophores. Miss Wakefield[13] has pointed out the probability of the existence of physiological varieties of *Stereum purpureum* some of which produce sporophores in artificial culture while under the same conditions others give rise only to vegetative mycelium.

INOCULATION EXPERIMENTS.

The results of a considerable number of inoculation experiments have been already reported. Those given below refer chiefly to inoculations which have not been previously described.

(1) *Inoculation Experiments with Plum trees.*

Inoculations with moist pieces of fructifications of *Stereum purpureum* taken from silvered Plum trees have continued to cause Silver-leaf in Victoria and Czar trees almost invariably. One "Czar" inoculated in this manner during 1911 shewed Silver-leaf in the spring of that year but during the summer it became leafless. No leaves were put forth in the spring of 1912 and during the autumn fructifications of *Stereum purpureum* developed in profusion on the main stem, from the level of the soil upwards. It is evident that this tree was killed by *Stereum purpureum*. The only failure to be recorded in connexion with these inoculations of Victoria Plums concerns three young bushes, the main stems of which were inoculated during December 1911. The foliage of the spring of 1912 was normal and no signs of Silver-leaf developed during the summer. Gumming, however, occurred at the places of inoculation. Two other bushes inoculated at the same time and in the same way produced silvered foliage the following spring. It is difficult to account for these failures. It is possible that for some reason not understood the mycelium failed to establish itself in the tissues with sufficient vigour to cause Silver-leaf and the affection may yet appear next spring, for in some other experiments its appearance has been delayed more than a year. The vigour of the sporophores used in these and similar inoculations was always tested by ascertaining whether control pieces would deposit spores under suitable conditions.

As would be anticipated, the silvering exhibited in the spring is usually the more extensive the longer the inoculation has been made.

When an inoculation is made during the autumn the inoculated branch sometimes dies during the winter, so that no leaves are put forth from the buds of this branch; silvering is however shewn by leaves developed on neighbouring branches. Inoculations made after July frequently do not result in silvering of the foliage until the following spring, but one inoculation of a Victoria Plum made during August 1912 caused silvering less than five weeks later. The leaves which became silvered were fully formed before the inoculation was made.

Salmon [11] has suggested the possibility of the existence of different strains or varieties of *Stereum purpureum*, some causing Silver-leaf and others being inactive in this way. The previous experiments of Spencer Pickering [9, 10] and myself [2] shew that there is probably nothing in the nature of specialised parasitism in the biology of this fungus. The mode of life of a wound parasite differs widely from that of an obligate parasite such as a Mildew or a Rust fungus, and there is less likelihood of a particularly specialised mode of nutrition being evolved in the case of a fungus which may live either saprophytically or parasitically than in one which is an obligate parasite. *Stereum purpureum* is, however, such a common saprophytic fungus upon various kinds of woody tissues that it is a matter of importance to ascertain if the fungus taken from tissues where it is not associated with Silver-leaf can produce this phenomenon in Plum trees. The following experiments in this connexion have been performed. Small portions of the sporophores of the fungus obtained from a dead Birch stump in the midst of a wood were placed in 12 branches belonging to two different Victoria plum trees, 4—5 years of age, during October 1911. In April 1912 when some of the buds opened, all of the 12 inoculated branches shewed silvered leaves. Many of the foliage buds of the inoculated branches, however, did not open, and the leaves which did develop, rapidly died so that the inoculated branches became bare. Eight of the inoculated branches were leafless by the end of April and some shoots which had meanwhile developed on the main stem of one tree two and a half feet below the level of the inoculations had become silvered. By the middle of June all the inoculated branches were leafless and the leaves of shoots which were being thrown up by the root stock were silvered. In this connection it is to be noted that all the Plum trees which have become seriously affected with Silver-leaf after inoculation have put forth numerous shoots both from the base of the stem and also from the root stock as though the trees were making strenuous efforts to free themselves from the disease. Fig. 6 is a

photograph of one of these trees taken in the early part of October 1912. It will be noticed that the tree is bare except for the silvered shoots which have developed on one side of the base. At the end of the month fructifications of *Stereum purpureum* began to arise on one side of the trunk of one of these trees throughout its whole length and were especially well developed at soil level on the side opposite that on which the silvered suckers had been thrown up. The tissues of the main stem were dead only on one side and the fructifications arose only from this region. The moist condition of the soil probably accounts for the particularly strong development of fructifications at this level. During the following month *Stereum purpureum* developed in much the same way on the main stem of the other Plum tree inoculated with pieces of sporophore obtained from a Birch stump. It is thus clear that these trees are dying with great rapidity. The trees were perhaps somewhat more severely inoculated than other trees inoculated with *Stereum purpureum* obtained from a silvered Plum tree but, taking this into consideration, they are undoubtedly being more rapidly killed than the latter. These experiments show that *Stereum purpureum* from such a source as a dead Birch stump may be at least as virulent in causing Silver-leaf as *Stereum purpureum* obtained from a silvered fruit tree. Indeed these results engender a suspicion that the fungus may be all the more aggressive for a change of "host."

Additional inoculations with the mycelium of *Stereum purpureum* cultivated in pure culture from spores have been followed by silvering of the foliage in a high percentage of cases. Thus 12 inoculations made in three "Victorias" with this mycelium during December 1911 all resulted in Silver-leaf the following spring. On the other hand 6 inoculations of a "Monarch" and 6 inoculations of an "Early Rivers" made during March 1912 have not hitherto resulted in Silver-leaf. These varieties are not so susceptible to Silver-leaf as the "Victoria" and may possibly resist the disease in these experiments. Two examples of the results of inoculating "Victorias" with the cultivated mycelium will be described in detail.

In one, six branches of a young bush tree were inoculated with cultivated mycelium in December 1911. At the end of April 1912, five of the inoculated branches bore silvered foliage, the other inoculated branch being leafless and apparently dead. Five uninoculated branches also carried silvered leaves. Large masses of gum were extruding both from the places of inoculation and from other points, whereas in control trees of the same variety there was only a trace of gumming. By the

middle of August all the inoculated branches were leafless. On one of the uninoculated branches which shewed silvered leaves in April a "midsummer shoot" had developed but none of its leaves became silvered.

In the other case three branches of a young bush tree were similarly inoculated in December 1911. At the end of April 1912 all the branches, inoculated and uninoculated, shewed silvered leaves and there was profuse gumming at the points of inoculation and at places on the main stem. By the middle of August the general appearance of the bush was about the same, but in this instance some of the leaves at the proximal end of a "midsummer shoot" were silvered.

In the paper of 1911 it was pointed out that of 38 branches of "Czars" and "Victorias" inoculated with the cultivated mycelium 10 became affected with Silver-leaf during that year. During 1912 eight of these inoculated branches were silvered and three of them were branches which did not carry silvered leaves the previous year. Some branches affected during 1911 have evidently recovered.

It was recorded in the same paper that a small number of branches of old Victoria plums inoculated with spores of *Stereum purpureum* had been followed by Silver-leaf. These successful spore inoculations were made during the summer of 1910 and silvering of the foliage resulted in 1911. In these cases spores were inserted into a T-shaped wound immediately after the latter had been made. No such success has followed any of the spore inoculations made in the same way during 1911 in branches of young Victoria trees.

A few weeks ago, another of the branches inoculated with spores during the spring of 1911 but which remained unsilvered during 1912, was cut off in order to trace the progress made by the fungus from the place of inoculation. It was found that a zone of discoloured wood covering about 90 degrees extended from an inch above the wound to 3½ inches below it, the diseased tissues being entirely surrounded by healthy wood. A similar branch cut off 18 months before shewed a zone of discoloured wood extending from three quarters of an inch above to 2 inches below the place of inoculation. It is therefore evident that the fungus has not made rapid progress recently.

On the other hand certain spore inoculations made in older wounds of branches of similar trees have been followed by Silver-leaf. These experiments will be described in detail. They concern two trees, one a "Czar" and the other a "Victoria," each being a half-standard 4—5 years old. In March 1911 six branches of each tree were broken across

so that they were half severed. The wounds thus made were exposed to the action of the weather for three weeks after which two branches of the "Victoria" were cut off above the wounds. At the same time spores of *Stereum purpureum* were inserted in the wounds of the twelve branches. The wounds were then covered with tinfoil and wool and each of the ten partly severed branches was bound in such a manner as to prevent it from being broken off by the wind. None of the inoculated branches shewed Silver-leaf during the summer of 1911 but during the spring and summer of 1912 three inoculated branches of each tree, *i.e.* six inoculated branches in all, carried silvered foliage. In regard to the Czar tree, two branches bore silvered leaves both above and below the places of inoculation; on the other branch only leaves below the wound were affected. Subsequently these three branches together with another inoculated branch which bore only healthy leaves, broke off at the places of inoculation on account of the heavy crop of fruit. In the case of the "Victoria" only one of the inoculated branches showed silvered leaves in April 1912 but two other uninoculated branches closely connected with it also exhibited Silver-leaf. Leaves of another inoculated branch the upper part of which had broken off, became silvered in June and immediately below it an uninoculated branch also carried silvered leaves. Later in the summer leaves of a third inoculated branch, the upper part of which had broken off, became silvered. It may be said here that several branches of other trees kept as controls also became partly severed and hung down during the summer but none of the leaves of these branches shewed Silver-leaf. One of the larger branches of the "Victoria" that carried silvered leaves and to which one of the inoculated branches was attached, was brought into the laboratory during October in order to ascertain what effect the inoculation had had upon the tissues. Passing downwards from the place of inoculation nothing but dead tissue was found until a lateral shoot bearing silvered leaves was reached two inches below, where about half the wood and bark, as seen in cross section, was discoloured in the same way as has been previously described in connection with my other investigations of Silver-leaf disease. Two inches further below, discoloured wood extended over about a quarter of the cross section. Hyphae were present in abundance in longitudinal sections of this zone of diseased wood. The lateral shoot which bore silvered leaves contained no discoloured wood immediately above its junction with the inoculated branch, hence the silvering of its leaves is to be probably attributed to the fungus present

in the diseased wood below. For the following reason as well as for the fact that the wounds were exposed for three weeks before inoculation it is impossible to say with certainty if the mycelium present in the discoloured wood was derived from the spores inserted at the time of inoculation. This wood is in direct continuity with the dead wood immediately below the place of inoculation but, unfortunately, pruning wounds are present on the branch and, as explained below, these prevent one from drawing a more definite conclusion as to the influence of the inoculation. Below an old pruning wound a certain amount of discoloured wood is invariably found, the discolouration being due to the action of various micro-organisms; the dead wood does not usually extend far back but in some cases I have found it two inches below the exposed surface. Hence it is uncertain how much of the tissue has been killed by the influence of pruning wounds and how much by the mycelium derived from the spores used at the time of inoculation. Silver-leaf has not developed in connection with the presence of pruning wounds on any of the other 70 or more young Plum trees used in the investigation and this is a strong argument in favour of the interpretation that the occurrence of Silver-leaf described above, together with the five other cases associated with it, was probably caused by the inoculation of a previously wounded branch with spores of *Stereum purpureum*. This interpretation supports the view that the fungus behaves as a wound-parasite in fruit plantations. Another possible interpretation of these results is that Silver-leaf developed as a consequence of the manner in which the trees were mutilated apart from any influence due to the insertion of the spores. However, Silver-leaf did not appear until the spring of 1912 and had the phenomenon been caused by pathological conditions outside the category of parasitic influences one might have expected it to become manifest during 1911. It is known from previous experiments that the fungus inserted in the form of spores takes considerable time to advance in the tissues sufficiently to cause Silver-leaf, so it is not surprising that the affection did not appear until 1912.

It is of interest to note that none of the control inoculations performed last year with pieces of dead sporophores of *Stereum purpureum* and with pieces of living sporophores of *Stereum hirsutum* and *Polystictus hirsutus* have been followed by Silver-leaf. Inoculations made during the summer of 1912 with *Stereum rugosum*[1] have not yet

[1] I am indebted to Miss Wakefield, of the Herbarium, Royal Botanic Gardens, Kew, for kindly sending me material of *Stereum rugosum*.

resulted in silvering. Two branches of a Czar tree inoculated with *Stereum hirsutum* were investigated in the laboratory in order to see if the fungus had developed in the tissues. I found that it had made considerable progress in each branch. Thus in one branch there was a zone of discoloured wood which extended from four inches below the point of inoculation to two inches above it, the average amount of diseased wood within these limits being about one third of the area of the cross section. Longitudinal sections of the discoloured zone shewed the presence of hyphae. It is thus evident that *Stereum hirsutum* grows with some facility in the tissues of Plum trees but until the present the phenomenon of Silver-leaf has not been associated with this fungus. Gussow[5] has discovered that *Polystictus versicolor* and *Bjerkandera adusta* grow well in living tissues of Apple trees, but their development in them has not yet been followed by silvering of the foliage. Münch[7] also has pointed out that *Schizophyllum commune, Stereum rugosum, Stereum purpureum, Stereum hirsutum* and other fungi cause extensive discolouration of the sapwood of old trees when inserted by inoculation.

It should be noted that even in control experiments in which a scalpel wound is made in a branch and is subsequently covered with tinfoil and wool, a small amount of discoloured wood is subsequently found around the wound. This is doubtless due to the fact that in outdoor experiments of this kind it is impossible to carry them out under conditions which are completely sterile.

Recovery of silvered Plum trees has already been recorded in previous papers by Spencer Pickering[10] and myself[2]. One of the Czar trees which was conspicuously silvered in 1911 in consequence of inoculation with a portion of a sporophore of *Stereum purpureum* shewed only the merest trace of affection during 1912. Two branches belonging to different trees which exhibited Silver-leaf in 1911 after inoculation with cultivated mycelium of *Stereum purpureum* were entirely unaffected during 1912. In this connection mention should be made of the complete recovery during 1912 of a Laburnum which was silvered in 1911 in consequence of inoculation with *Stereum purpureum*. These cases of recovery may be considered to be due to a check in the growth of the fungus, possibly on account of active resistance on the part of the host.

(2) *Inoculation Experiments with Apple trees.*

Numerous inoculations of young Apple trees have been made with different kinds of material of *Stereum purpureum*. Sporophores from silvered Plum trees, cultivated mycelium, and spores, have been placed many times during 1911 and 1912 in branches of the following varieties of Apple trees: "Lane's Prince Albert," "Lord Suffield," and "Stirling Castle," but until the present only one such inoculation has resulted in Silver-leaf. In this particular case a branch of a bush "Lord Suffield" was inoculated in May 1912 with a piece of sporophore obtained from a silvered Victoria Plum tree and silvering of four leaves at the top of this branch, about two feet above the place of inoculation, became apparent in August. Three other branches of the same tree and four branches of another tree of the same variety were similarly inoculated at the same time but the foliage remained normal throughout the summer. It is evident that in these experiments Apple trees of the varieties named have been much less susceptible to Silver-leaf than are Plum trees. These results may be contrasted with those of Gussow[5] who finds that Apple trees in Canada are readily susceptible to Silver-leaf when inoculated with *Stereum purpureum*. Güssow does not mention the names of the varieties of Apple trees used for the purpose of inoculation. They may have been different from those used by me and this may account for the variation in results.

Upon examining inoculated branches the foliage of which has remained unaffected it is seen that the fungus has made some progress in the tissues. Thus a branch of a "Lane's Prince Albert" inoculated in February 1911 shewed in November 1911 a small zone of discoloured wood that extended two inches below the place of inoculation and an inch above it, the amount of affected wood being relatively much less than is usually the case in similar inoculations of Plum trees.

(3) *Inoculation Experiments with other kinds of Fruit Trees.*

A considerable number of young Cherry trees, chiefly of the "Florence" variety, were inoculated with *Stereum purpureum* in various forms during 1910—11 but none of these trees have become affected with Silver-leaf. Profuse gumming invariably followed the inoculations when pieces of natural sporophores from a silvered Plum tree or cultivated mycelium were used. At a later date a large number of the inoculated branches died rapidly but neighbouring branches remained

unaffected. Investigation shewed that fungus mycelium was abundant in the branches killed in this manner. This mycelium probably belonged to *Stereum purpureum* but although it frequently killed the inoculated branches with rapidity it did not pass thence into other branches.

A Gooseberry bush of the variety known as "Whinham's Industry," inoculated during 1911 with pieces of natural sporophore of *Stereum purpureum*, became silvered during 1912.

OBSERVATIONS ON THE MANNER OF INFECTION AND TREATMENT OF THE DISEASE.

The evidence brought forward in the present paper supports the view that in causing Silver-leaf disease in fruit plantations, *Stereum purpureum* behaves as a wound parasite, the disease being spread from one tree to another chiefly by means of spores. There is usually no lack of unprotected wounds on fruit trees and if *Stereum purpureum* is allowed to fructify in a plantation of Plum trees it is likely that Silver-leaf disease will become increasingly prevalent there. Even in fruit gardens that are well managed in other respects, fructifications of *Stereum purpureum* are sometimes allowed to develop with impunity. It cannot be too strongly urged that all tissues on which the sporophores of this fungus appear in fruit plantations should be destroyed. Experiments indicate that *Stereum purpureum* taken from material such as a dead Birch stump is equally as effective in causing Silver-leaf as *Stereum purpureum* taken from a silvered Plum tree, hence no quarter should be extended to the fungus in fruit plantations on whatever substratum it may be found.

Where Silver-leaf has appeared in a Plum plantation experience has shewn that benefit is derived by cutting out affected branches, but in order that this operation may be successful, care must be taken to cut back below the region of discoloured wood. Cases of recovery of trees which are slightly silvered are not infrequent but I see no reason to alter the suggestion previously made that Plum trees which are badly silvered and are beginning to die back, should be destroyed. Reference was made in my earlier paper to the danger of making wood piles in fruit plantations. The prevention of such accumulations is one of the first principles of plant sanitation.

The large amount of pruning and thinning out to which fruit trees are necessarily subjected may be indirectly responsible to some extent

for the malady. Even with rapidly growing Plum trees it is exceptional for a callus to grow sufficiently to protect fully the severed end of a branch from micro-organisms possessing dangerous tendencies.

Grease-banding when not properly carried out is, I think, also an influence which favours the development of Silver-leaf disease. Where the grease has been placed directly on the tree or has soaked through the band, one often sees that the bark becomes torn and dead in places. Such broken tissues offer facility for the development of *Stereum purpureum* and I have seen silvered trees on the torn bark of which *Stereum purpureum* was growing in profusion. Both for this reason and for others which are well known to fruit-growers, the grease should not be placed directly on the tree, the bands should be such that the grease cannot penetrate to the bark, and their position should be altered somewhat each autumn so that the same area of bark is not covered in successive years.

No outbreaks of Silver-leaf have been seen in which it was clear that infection had first occurred in the roots. If a tree that is badly silvered is dug up, a white mycelium is usually found attached to the roots but I have not yet seen any evidence of a tendency for it to spread outwards in the soil. This mycelium is readily traced into the discoloured parts of the wood. Of course if the trees were planted so closely that their root systems interlaced there would be abundant opportunity for infection to occur in a subterranean manner.

It has been suggested by fruit-growers that application of Ferrous Sulphate to the roots of silvered Plum trees is a means of cure, but Spencer Pickering[10] tried this method of treatment on a considerable scale at the Woburn Experimental Fruit Farm a few years ago without success.

A writer in the *Gardeners' Chronicle*[1] has recently given details of a similar method of treating silvered trees which he has found successful. The roots of silvered Plum trees were treated during 1910 and 1911 with heavy dressings of Ferrous Sulphate together with either farm-yard or complete artificial manure. In 1912 the trees thus treated shewed great improvement and one was quite cured. Only a small number of trees were treated in this way so that until the method has been tried on a larger scale it is difficult to pronounce an opinion upon it. The writer of the note was certainly hopeful of this method of treatment.

During the last two years I have had the opportunity of observing some experiments on a method of treating silvered Plum trees with

Ferrous Sulphate in a different manner. These experiments have been carried out near Wisbech by Mr E. Neaverson, who has kindly placed the following data at my disposal. Forty-nine silvered Victoria Plum trees about 20 years of age were treated in the following manner during August 1910: a hole was drilled in the trunk of each tree about three feet from the ground, the hole extending in most cases only to the outer part of the wood; about an ounce of Ferrous Sulphate was inserted in each hole which was afterwards closed with a cork bung. Of the forty-nine trees thus plugged, thirty-seven were slightly affected with Silver-leaf and the remainder were badly diseased at the time of treatment. An examination of the trees in August 1912, *i.e.* two years after treatment, shewed that twelve of the thirty-seven trees slightly infected in 1910 had recovered and were free from Silver-leaf, while none of the twelve trees badly diseased in 1910 had recovered. Thus taking all the plugged trees into consideration 25 per cent. of them had recovered while 34 per cent. of those only slightly affected have been restored to health. It is known that trees lightly attacked by Silver-leaf sometimes recover without treatment, hence it is difficult to lay much stress on the above figures, and in this particular garden one of five untreated silvered trees did recover during the period. On the other hand five of the slightly affected trees which were treated became so badly diseased during the summer of 1911 that they were felled in the autumn. Thus the above method of treating silvered Plum trees, even when only slightly attacked, does not at present appear to give much promise of success. The method is an empirical one, but it was obvious that the method should be seriously considered on account of the faith retained in it by fruit-growers. It is likely that one reason for the use of Ferrous Sulphate as a cure for Silver-leaf lies in the fact that the disease is sometimes confounded with Chlorosis, some cases of which are alleviated by applications of Iron compounds. Ferrous Sulphate placed in the trunk of a tree as in the experiments described above would certainly have a poisonous effect on mycelium present in the wood immediately around the place of application, but it is doubtful whether its influence would be extensive[1].

[1] While this paper was passing through the press a note by Miss Baker on a new treatment for Silver-leaf disease appeared in the *Annals of Botany* for January, 1913. Miss Baker applied, both internally and externally, a concentrated aqueous extract of deliquescing fruit bodies of *Coprinus* to a silvered branch of a Victoria Plum and found that two years later this branch became almost entirely free from Silver-leaf and put forth vigorous new growth. In this note reference is made to the effect of the treatment on one tree only, so the results of its application on a large scale will be awaited with interest.

As indicating the rapid way in which Silver-leaf may spread amongst Victoria Plum trees it may be said that in the garden mentioned above, 17 trees out of 75 which were healthy in 1910 have since become affected by the disease. *Stereum purpureum* was present in abundance on silvered trees in this garden.

General Considerations and Conclusions.

It has been shewn that Silver-leaf is a pathological condition of widespread distribution, the chief cause of the malady in the fruit plantations of this country being the fungus *Stereum purpureum*. This view is held also by Percival[8], Spencer Pickering[9, 10], and Güssow[5]. Examples of silvered foliage have, however, come under observation which, in my opinion, cannot be attributed to the action of *Stereum purpureum*. It is unlikely that the silvering of the leaves of seedling Plums and of such a plant as the White Dead Nettle is caused by this fungus. I look upon Silver-leaf as a general pathological phenomenon which may be caused in various ways, although at present only one of these agents, the fungus *Stereum purpureum*, is known with certainty. It appears likely that Silver-leaf may be caused also by physiological disturbances which are not connected with the action of any parasitic organism as has been suggested by Massee[6a]. It will be remembered that *Stereum purpureum* living in a branch of a Victoria Plum tree may produce silvering of the leaves a considerable distance beyond the region attained by the mycelium; such action at a distance may be due to a disturbance in the transpiration current induced by the presence of the fungus below, though until the present I have been unable to confirm Percival's view[8] that the disturbing agent is an oxidase which is secreted by the fungus. Similar disturbances in the transpiration current may possibly arise from causes not associated with the action of parasitic organisms and the same phenomenon of Silver-leaf may thereby be induced. It is by no means unusual in the pathology of plants and animals for the same kind of morbid manifestation to appear as the result of different pathogenic agents, and in considering Silver-leaf a general pathological phenomenon, I look upon it somewhat in the same way as upon such a condition as, for example, hypertrophy which may be induced both by the action of various parasites and by disturbances in metabolism unconnected with the action of micro-organisms.

The views here expressed on Silver-leaf may possibly harmonise the opinions of those who have considered it to be due to *Stereum*

purpureum with the views of those who have placed it in the category of diseases which are not caused by the action of parasitic organisms. Thus Sorauer [12] in writing of the phenomenon known as "Milchglanz" in Germany, which is doubtless identical with Silver-leaf, describes it under the section devoted to non-parasitic diseases in his *Handbuch der Pflanzen-Krankheiten.* Delacroix [4], who speaks of it as "le plomb" in France, also treats of the disease in the same category.

The manifestation of Silver-leaf depends, I think, partly upon leaf-structure. It is well known that certain varieties of fruit-trees exhibit this phenomenon much more than others and cases have been already mentioned in which Silver-leaf has not resulted although *Stereum purpureum* has made considerable progress in the tissues. I have seen Apple and Beech trees which have been killed by *Stereum purpureum* in all probability, but with which the phenomenon of Silver-leaf has not been associated. Thus just as the phenomenon of Silver-leaf cannot always be attributed to *Stereum purpureum*, so the destructive influence of *Stereum purpureum* is not invariably accompanied by this peculiar affection of the leaves.

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EXPLANATION OF PLATES XI AND XII.

Fig. 1. Upper part of a silvered Victoria Plum tree, one branch of which is dead and bears fructifications of *Stereum purpureum*.

Fig. 2. Fructifications of *Stereum purpureum* on a dead branch of a Victoria Plum tree.

Fig. 3. Lower part of the trunk of a silvered Victoria Plum tree shewing fructifications of *Stereum purpureum* which developed shortly after the upper part of the tree was cut off.

Fig. 4. Cross section of a branch of a silvered Transparent Gage tree shewing diseased and healthy wood. Twice natural size.

Fig. 5. A Blenheim Orange Apple tree that has died after being regrafted with scions of "Grenadier" or "Jubilee." The scions also have died and the stock bears fructifications of *Stereum purpureum*.

Fig. 6. A Victoria Plum tree as seen one year after inoculation with *Stereum purpureum* taken from a Birch stump. The tree is dying rapidly. Fructifications of *Stereum purpureum* have appeared on the main stem of this tree since the photograph was taken.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

STUDIES IN MILK RECORDS: THE INFLUENCE OF FOETAL GROWTH ON YIELD.

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Introductory.

In a previous paper on the "Interpretation of Milk Records[1]" the following points were dealt with:

I. Selection of a figure definitive of a cow's milking capability.

II. The influences affecting such a figure.

The conclusions there reached may be summarised as follows:

(*a*) The usual methods of describing a cow by her total yield per calendar year, per lactation, or per average week are inconvenient both for practical breeding on a large scale and for definite enquiry on the inheritance of milk yield or its possible correlation with other characters. The figures are inconvenient because a variety of circumstances which affect them must be stated for every individual case before such figures can be reliable. Chief of these circumstances are age of cow, length of lactation, number of weeks dry before calving, interval between calving and subsequent service, and time of year of calving.

(*b*) It is therefore advisable to adopt some additional descriptive figure affected by the minimum number of these influences, and to estimate as accurately as is possible the effect of those influences under which it falls.

(*c*) The maximum day-yield three times reached or exceeded[2] is chosen as being the most satisfactory figure, and termed the "Revised Maximum," or R.M.

[1] *Journal Royal Agricultural Society*, 1912, p. 153.

[2] *I.e.* the highest figure common to three entries in record book, whether yields are recorded daily or weekly. What is aimed at is really the correct maximum day-yield: the stipulation that it should have appeared three times is merely to avoid the errors associated with a single sampling.

(*d*) This Revised Maximum bears a close relationship to the total yield of a normal lactation, and shews rather less variation than that total. The following constants were obtained:

Correlation coefficient between revised maxima and normal lactation totals							$+0{\cdot}844 \pm 0{\cdot}005$
Coefficient of variation.	Totals for normal lactations			...	...	...	$25{\cdot}72 \pm 0{\cdot}37$
,,	,,	,,	all	,,	...	...	...	$31{\cdot}69 \pm 0{\cdot}32$
,,	,,	R. M. for normal		,,	...	...	...	$24{\cdot}77 \pm 0{\cdot}36$
,,	,,	,,	all	,,	...	...	...	$26{\cdot}44 \pm 0{\cdot}25$

(*e*) The Revised Maximum is outside two of the most active external influences, namely length of lactation, and time of service, and it is suggested that general environment has a minimum effect on it.

(*f*) A cow can usually be judged within a few weeks of calving, since normal lactation totals can be estimated from the Revised Maximum with considerable accuracy.

(*g*) The influence of length of rest before calving is negligible as regards the Revised Maximum. The latter has to be corrected however according to the age of cow, and season of year of calving.

(*h*) By a simple but provisional scheme of correction the mode of the extreme differences found from year to year in the Revised Maxima of individual cows can be reduced from 7 quarts to $3\frac{1}{2}$ quarts.

It will be noticed that the Revised Maximum is referred to above as being outside the influence of:

(1) Length of Lactation, and

(2) Time of Service of the cow.

This contention is certainly approximately true, though in some cases the mean Revised Maximum was found to increase very slightly with length of lactation. A possible explanation of this was discussed in the paper referred to. It is thought however that the statement regarding time of service requires fuller proof than has yet been obtained, and the present paper gives the result of further work on the point.

Time after calving at which maximum yield first occurs.

The question as to whether the maximum day-yield can be influenced by the date of service must be dependent on whether that maximum is reached by a cow before or after the growth of the young calf within her has caused any alteration in her output of milk.

It is therefore necessary to determine firstly the time after calving at which the maximum occurs, and secondly the time after service at which foetal growth begins to reduce the milk yield.

In a preliminary examination it was found that the large majority of cows reached their maximum day-yield within a few weeks of calving, but that some of those calving about the beginning of the year did not do so until turned out to grass in the spring.

The 1421 records that were examined for this point were consequently divided into five groups according to whether the cows calved during (1) April to November inclusive, (2) December, (3) January, (4) February, or (5) March. Table I gives results obtained in actual number of cows: Table II in percentages.

TABLE I.

	Number of cows reaching maximum day-yield during :—								
e of ing	1st—4th week after calving	5th—8th week after calving	9th—12th week after calving	13th—16th week after calving	17th—20th week after calving	21st—24th week after calving	25th—28th week after calving	29th—32nd week after calving	Total number of cows
to / mber	580	249	21	3	4	1	3	1	863
mber	77	38	14	1	5	6	0	0	141
ary	55	38	5	17	17	4	0	0	136
ary	73	26	31	43	3	0	0	0	176
h	24	33	40	7	0	0	0	0	104
	809	384	111	71	29	11	3	1	1419
							2 cows after 32nd week		2
									1421

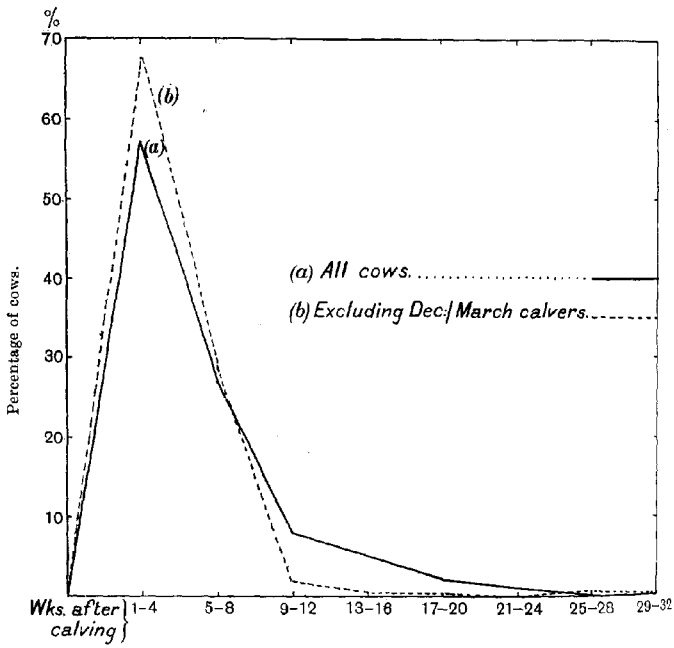
TABLE II.

Percentage of cows reaching maximum day-yield during :—								
1st—4th week after calving	5th—8th week after calving	9th—12th week after calving	13th—16th week after calving	17th—20th week after calving	21st—24th week after calving	25th—28th week after calving	29th—32nd week after calving	
67·5	29	2	·5	·5	—	·5	—	100
55	27	10	·5	3·5	4	—	—	100
40·5	28	3·5	12·5	12·5	3	—	—	100
41·5	15	17·5	24·5	1·5	—	—	—	100
23	31·5	38·5	7	—	—	—	—	100
57	27	8	5	2	1	—	—	100

It will be seen that 84 °/₀ of the total number of cows reached their maximum day-yield by the 8th week after calving, 92 °/₀ by the 12th week, and 97 °/₀ by the 16th week. Three-quarters of the 8 °/₀ that had not reached their maximum by the 12th week were January and February calves, leaving only 2 °/₀ for cows calving during the remainder of the year.

Taking the January and February calvers by themselves, only 72 °/₀ of the former gave a maximum before the 12th week, but 97 °/₀ had given it by the 20th week. February calving cows alone shew corresponding figures of 74 °/₀ for the 12th week, and 100 °/₀ for the 20th.

The influence of these "new-year" calvers is shewn in the following diagram, Fig. 1, which gives the curve of *all* cows compared with that obtained when December/March calving cows are excluded.



Time at which first maximum is reached.

Fig. 1.

In Fig. 2 the curves for each group in the previous table have been superimposed upon one another according to their calendar dates, and this arrangement brings out the fact that the delayed maxima are in every case ultimately reached about the same season of the year, namely April/May, when the cows respond to the extra stimulus of abundant and succulent green food.

Two cows out of the 1421, that gave their maxima after the 32nd week, have not been included in the table. One of these calved in July, and one in August, and both gave their maxima the following April when turned out to grass, in the 39th and 42nd week after calving respectively.

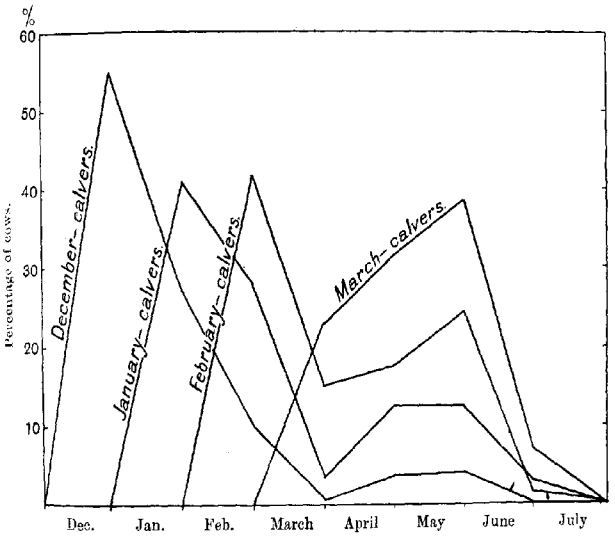


Fig. 2.

Influence of time of service.

247 records of cows calving in May and June were examined. They fell into the following groups:

	Time of service	*No. of records*
Group I.	5th—8th week after calving	51
,, II.	9th—12th ,, ,,	55
,, III.	13th—16th ,, ,,	49
,, IV.	17th—20th ,, ,,	40
,, V.	21st—24th ,, ,,	34
,, VI.	after 36th ,, ,,	18
	[All calved between May 1st—June 30th]	247

The average daily yields in each group for the first 36 weeks after calving are given in Table III. The figures representing first and second week's yield are not reliable, as some cows were suckling calves for all or part of that time.

TABLE III.

Week after calving	I Served 5th—8th week after calving	II Served 9th—12th week after calving	III Served 13th—16th week after calving	IV Served 17th—20th week after calving	V Served 21st—24th week after calving	VI Served after 36th week after calving
	Quarts	Quarts	Quarts	Quarts	Quarts	Quarts
1	[11·6]	[9·6]	[6·7]	[9·3]	[9·9]	[8·1]
2	[13·9]	[13·3]	[12·2]	[12·1]	[12·2]	[10·4]
3	14·9	14·1	13·3	13·9	13·2	12·9
4	14·8	14·1	14·1	14·1	13·3	13·4
5	14·8	14·2	13·8	14·0	13·2	13·3
6	14·6	14·2	13·7	13·9	13·2	13·1
7	14·1	13·9	13·4	13·6	13·1	12·6
8	13·8	13·4	13·2	13·2	12·5	12·3
9	13·5	13·1	12·4	13·0	12·4	11·9
10	13·2	12·7	11·8	12·7	12·0	11·7
11	12·8	12·4	11·5	12·4	11·6	11·4
12	12·6	12·3	11·3	12·1	11·2	11·1
13	12·4	12·1	11·1	12·0	10·7	10·9
14	12·2	11·6	10·8	11·7	10·5	10·7
15	11·9	11·3	10·3	11·3	10·0	10·3
16	11·5	11·1	10·0	11·1	9·8	9·8
17	11·1	10·7	9·8	10·8	9·6	9·7
18	10·9	10·3	9·4	10·5	9·4	9·2
19	10·6	10·3	9·3	10·1	9·4	9·3
20	10·3	10·1	9·0	9·8	9·4	9·4
21	9·9	9·7	8·8	9·5	9·0	9·1
22	9·5	9·4	8·6	9·4	8·9	8·5
23	9·0	9·2	8·6	9·3	8·9	8·1
24	8·7	9·1	8·4	9·1	8·7	8·2
25	8·4	8·8	8·3	9·0	8·6	8·1
26	8·0	8·5	8·1	8·6	8·4	7·8
27	7·6	8·3	7·9	8·5	8·3	7·7
28	7·1	7·9	7·6	8·3	8·0	7·6
29	6·6	7·6	7·4	7·9	7·9	7·5
30	6·1	7·3	7·1	7·8	7·8	7·7
31	5·6	6·9	6·9	7·4	7·8	7·2
32	5·2	6·4	6·6	7·4	7·7	7·1
33	4·7	5·8	6·2	7·2	7·6	7·0
34	4·2	5·1	5·8	6·9	7·4	6·9
35	3·0	4·4	5·3	6·4	7·3	6·7
36	2·3	3·8	4·8	6·1	7·2	6·3

This somewhat unwieldy mass of figures was concentrated by dividing the 36 weeks into nine periods of four weeks each, and obtaining the average for each period. The figures are given in Table IV.

TABLE IV.

Week after calving / Periods	I Served 5th—8th week after calving	II Served 9th—12th week after calving	III Served 13th—16th week after calving	IV Served 17th—20th week after calving	V Served 21st—24th week after calving	VI Served after 36th week after calving
3rd and 4th	14·8	14·1	13·7	14·0	13·3	13·2
5th—8th	14·3	13·9	13·5	13·7	13·0	12·8
9th—12th	13·0	12·6	11·8	12·6	11·3	11·5
13th—16th	12·0	11·5	10·6	11·5	10·3	10·4
17th—20th	10·7	10·4	9·4	10·3	9·5	9·4
21st—24th	9·3	9·3	8·6	9·3	8·9	8·5
25th—28th	7·8	8·4	8·0	8·6	8·3	7·8
29th—32nd	5·9	7·1	7·0	7·6	7·8	7·4
33rd—36th	3·6	4·8	5·5	6·7	7·4	6·7

By calling the maximum 100 for every group, we get a more comparable series of figures shewing average yield as percentage of group maximum.

TABLE V.

Week after calving / Periods	Time of service I	II	III	IV	V	VI
3rd and 4th	100	100	100	100	100	100
5th—8th	97*	99	99	98	98	97
9th—12th	88	90*	86	90	89	88
13th—16th	81	82	77*	82	**77**	79
17th—20th	73	73	69	73*	**71**	71
21st—24th	63	66	63	66	67*	64
25th—28th	**52**	59	58	61	63	60
29th—32nd	**40**	**50**	**51**	55	59	56
33rd—36th	**23**	**34**	**40**	**47**	56	51
37th—40th	—	—	—	—	48	47
41st—44th	—	—	—	—	**35**	45

* Period of service.

Time after which foetal growth appears to influence yield entered in dark figures.

It would seem that in no case has foetal growth reduced yield (as compared with that shewn for the same period by groups where service has not occurred) sooner than 12/16 weeks after service.

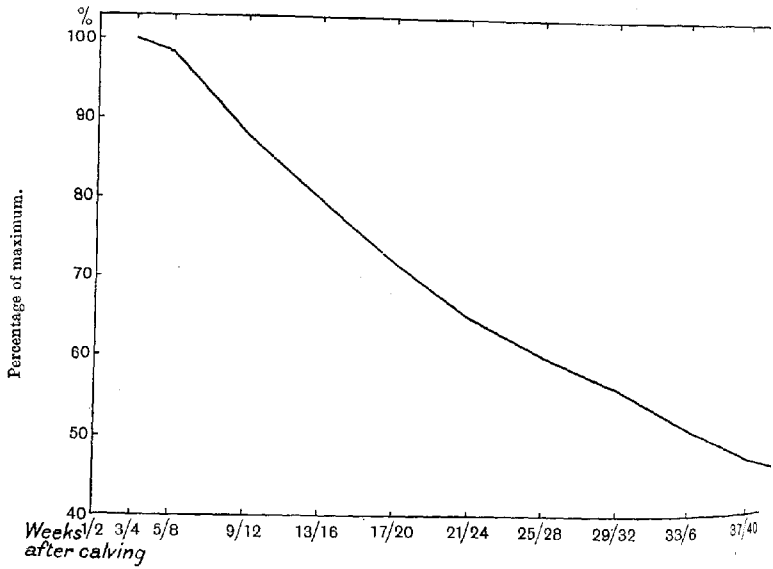
In the case of groups I, II, and V it appears to have had no influence for 16/20 weeks.

Since then 12 weeks at the very least (and probably 16/20 weeks) must elapse between *service* and any fall in yield due to foetal developement, and since 97 °/₀ of cows were found to have reached their maximum day-yield within 16 weeks of *calving*, and 99 °/₀ within 20 weeks, the chances of the "Revised Maximum" being affected by time of service are seen to be very slight.

It is evident that the fall in yield due to foetal growth must be clearly distinguished from the fall that normally occurs in the absence of gestation.

Fall of milk-yield where pregnancy does not occur.

Fig. 3 gives a curve illustrating fall of milk-yield in the absence of gestation. Up to 24th week after calving it is compiled from the average of all groups, after which it follows Group V.



Fall in milk-yield of non-pregnant cows.

Fig. 3.

The cause of this gradual failing of the milk-supply does not at present seem very clear.

It is now fairly well established that the growth of the mammary gland before parturition is brought about by an internal secretion elaborated by the foetus or placenta, and that the cessation of that secretion that necessarily coincides with the expulsion of the foetus causes the breaking down of the tissue formed and the consequent production of milk[1].

The institution of the milk-flow seems therefore dependent on a single and non-recurring stimulus. But having thus been once suddenly set up, it neither falls with equal rapidity, nor does it remain in constant activity, but gradually diminishes through a period of at least 6/9 months. Indeed if gestation does not intervene the lactation may be very greatly prolonged, though for commercial reasons this does not of course often occur with cows. On examining records for the present paper one cow was noticed which was sold in milk 163 weeks after calving. During this period she gave 2433 gallons, and was yielding seven quarts a day at the time of sale.

It is said that removal of the ovaries will cause lactation to continue for several years, but definite proof seems wanting that the same prolongation could not have been obtained on the same cows by constant and careful milking combined with high feeding. May not the theory have arisen through the operation removing not only the ovaries but also all possibility of gestation[2]?

Such cases seem to involve katabolic processes out of all proportion to the anabolism brought about by the foetal hormone before parturition, and at present we know little of any additional mechanism for maintaining the milk flow. Two points in this connection however must be mentioned.

Firstly, the removal of the products of the gland is of primary importance in prolonging its activity. It is a well-known fact that failure to empty the udder will very soon cause a cow to "dry-off." It is also supposed that the sucking of the teat[3] or other mechanical means

[1] v. Marshall, *Physiology of Reproduction*, 1910, p. 583.

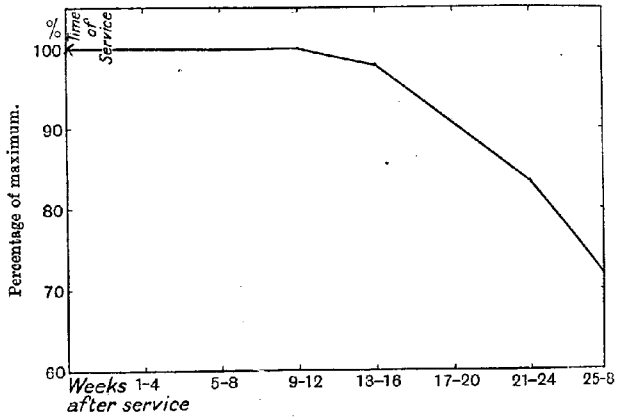
[2] In other cases the ovarian influence is established, as in the growth of the mammary glands at puberty in woman, which does not take place if the ovaries have been previously removed. It must be admitted that from this and other observations their influence certainly appears to be anabolic, and so in a contrary direction to milk-flow.

[3] Knot, "Abnormal Lactation," *American Medicine*, vol. II. 1907. Cases given of virgin girls who were nurses secreting copious supplies of milk as a consequence of allowing infants to apply this excitation. Also cases in which suckling occurred in a bull, a male goat, a wether, and in men. Quoted from Marshall (*loc. cit.*).

of withdrawing the milk[1] has a stimulating effect on secretion. The cessation of this excitation may also aid in the drying-off of a cow when milking is discontinued.

Secondly, it has recently been shewn[2] that certain organs of the body produce hormones possessing galactagogue action. The most marked results are obtained from the pituitary body, but the pineal body, corpus luteum, involuting uterus, and lactating mammary gland itself all yield extracts which, when injected into the blood-stream, cause an increased secretion of milk.

This increase, in cows[3] and goats[4] at any rate, seems to be followed by a corresponding decrease, so that the total activity of the gland is not changed. It seems probable therefore that these hormones are more directly concerned with the regulation than with the maintenance of secretion.



Fall in milk-yield due to foetal growth.
For 28 weeks after service.

Fig. 4.

[1] Marshall (*loc. cit.*) gives a case where secretion was induced by repeated attempts at milking. A mare which had never had a foal could be made to yield milk by this means at any time for years.

[2] Mackenzie, *Quart. Journ. Exper. Physiology*, IV. 4, 1911. Schäfer, *Proc. Royal Society*, B. LXXXIV. 1911.

[3] Gavin, *Quart. Journ. Exper. Physiology*, VI. 1, 1913.

[4] Hammond, *Quart. Journ. Exper. Physiology*. In course of publication.

Fall of milk-yield due to foetal developement.

By taking the excess of reduction shewn by groups where service occurred over that shewn by Group V, we get the following curve of diminution due to foetal growth *alone*. The data are not extensive and only the first 28 weeks after service are taken, but it is curious to note that once the reduction begins, the rate of fall due to this cause is very similar to that found where there is no gestation.

Where gestation occurs, the reduction of a cow's milk-yield is of course represented by the sum of the curves in Figs. 3 and 4, the latter coming into operation according to the time after calving at which service takes place.

These investigations are being undertaken on behalf of Lord Rayleigh and the Hon. E. G. Strutt with data accumulated by them during the last 20 years. For any deficiencies in method or treatment of the material, however, the author is alone responsible.

THE PARTIAL STERILISATION OF THE SOIL BY MEANS OF CAUSTIC LIME.

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THE practical advantages accruing from the use of lime are now generally recognised, but on account of the complexity of the changes induced it is by no means easy to determine, in the case of a field soil, the exact extent to which one effect or set of effects has contributed to the general improvement of the soil in relation to its crop-producing power.

The changes in the physical properties of a soil are accompanied by better drainage and increased aeration; the chemical action of lime leads to a liberation of plant food and the neutralisation of any acids present, and thus produces conditions more favourable to plant and bacterial growth.

Little distinction has hitherto been drawn between the effects of different calcium compounds on the soil, although such differences are plainly evident in agricultural practice. Indeed, very often the sole explanation met with is the one advanced by Boussingault, that the superiority of caustic lime over chalk lies in the extremely fine state of division in which the former is deposited as carbonate in the soil. It is the purpose of this preliminary account to indicate how essentially different this action may be and to attempt to account for such results as are frequently obtained in practice.

It has been frequently shown that when soil is treated with mild antiseptics or subjected to high temperatures, an increase of fertility ensues, and this has been attributed by Russell and the author[1] to a depression or destruction of soil protozoa, with a subsequent increase in bacterial growth, and production of available plant food. This effect is

[1] This *Journal*, vol. III, Part 2, 1909.

induced by a number of chemical compounds varying largely in composition and constitution, but fulfilling the essential requirement that the substance used should be capable of destroying protozoa, of volatilising from the soil or of being rendered innocuous to bacterial and plant growth at some subsequent period. Viewed in this light a similar effect ought to be obtained by applications of quick lime in quantities sufficient to destroy protozoa, while any excess would be converted sooner or later into carbonate, and normal growth be made possible.

Chemical and bacteriological analyses of two different soils to which various additions of calcium oxide had been made showed the correctness of this assumption.

Both these soils contained sufficient carbonate (2—3 per cent.) for all ordinary requirements, and indeed, the addition of further doses of calcium carbonate or even small quantities of oxide was without effect: when, however, the amount of oxide used was large enough to kill the larger forms of soil protozoa the general behaviour of the samples agreed closely with that of soils subjected to high temperatures or treated with different antiseptics.

When lime was applied in the proportion of 0·25—1·0 per cent. of the weight of soil, it was possible to detect an initial depression in the numbers of bacteria, and this effect persisted for a period the length of which appeared to be determined partly by the quantity of lime applied and partly by the character of the soil. In the case of a poor soil and an addition of 1·0 per cent. of calcium oxide, this depression was still evident after 3—4 months, whilst with a rich garden soil there was no trace of inhibitive action at the end of 3 weeks. Conversion of the oxide into the carbonate was followed by a period of active bacterial growth with increased production of plant food.

Pot experiments were made with these two soils and the results agree, in the main, with those obtained by bacteriological and chemical analyses of the soils.

Experimental.

In March, 1911, a poor unmanured soil from Hoos Field at Rothamsted, containing 3 per cent. calcium carbonate, was passed through a 3 mm. sieve and filled into bottles in lots of 800 grams. One lot served as control and the others received 0·1, 0·5 and 1·0 per cent. calcium oxide respectively. The soils were moistened

so that the water content was about 18 per cent. and the bottles were then set aside at room temperature. Quantitative analyses for bacteria were made on nutrient gelatine, and small portions of soil were carried over to hay infusion to test for the presence of protozoa.

The results of these counts are set out in Table I together with the occurrence of protozoa. Estimations of nitrogen as ammonia and nitrates were made at the close of the experiment and showed that in all cases except that in which the soil received 1·0 per cent. calcium oxide, there had been complete conversion of ammonia to nitrates. In this one case ammonia had accumulated to the extent of 22 parts per million of the dry soil but it is not apparent whether this was formed by direct chemical decomposition of nitrogen compounds or to the action of bacteria.

TABLE I. *Showing the effect of additions of Calcium Oxide on numbers of Bacteria and Ammonia and Nitrate production.*

Soil and Treatment	Protozoa in soil after treatment	Bacteria (in millions) per gram of dry soil after					Ammonia and Nitrate parts per million of dr soil after 250 days		
		7 days	18 days	140 days	200 days	250 days	Ammonia	Nitrate	T
Untreated soil	Vorticella, Colpoda, Amoebae and Monads	12·1	11·55	—	—	12·9	nil	17·1	1
Soil + 0·1 % CaO	Vorticella, Colpoda, Amoebae and Monads	8·4	15·3	16·5	15·3	15·2	nil	19·6	1
Soil + 0·5 ,,	Amoebae and Monads	2·4	18·3	125·3	157·5	70·8	1	52·1	5
Soil + 1·0 ,,	,, ,,	1·1	3·4	61·3	444·0	300·4	21·5	7·8	2

Just as in the case of soils treated with toluene, chloroform, and similar antiseptics, so in this case the death of the larger protozoa is accompanied by increased bacterial growth[1] and plant food production. The bacterial content of the heavily limed soils is considerably higher than is usually obtained by the use of mild antiseptics and might be attributed to a combined partial sterilisation effect and a more or less far-reaching chemical decomposition of organic nitrogen compounds in the soil. Further evidence of this action will be adduced later on.

[1] Since going to press, the author has found similar increases in bacterial numbers recorded by Fischer (*Landw. Versuchs-Stat.*, 1909, **70**, p. 335), who attributes them to stimulation effects.

It would appear from these data that caustic lime exercises initially a distinct depressive action on the growth of bacteria when applied to the soil in quantities greater than 0·1 per cent., but the intervals between the times of analyses in the early stages are too long to allow of any deduction as to the rapidity of the removal of caustic lime in this case.

In order to ascertain the rapidity of this change to carbonate, a second series of experiments was started in November 1911, with a similar arable soil and also a rich garden soil containing 2 per cent. calcium carbonate. The treatment was similar to that of the previous series, but estimations of bacteria were made during the period in which initial depression and subsequent recovery of bacterial growth were thought to take place.

The results contained in the following Table show how very different these soils are in their behaviour to treatment.

TABLE II. *Showing the effect of additions of Calcium Oxide to Arable and Garden Soils.*

Soil and Treatment	Protozoa in Soil after treatment	Bacteria (in millions) per gram of dry soil after			Ammonia and Nitrates, parts per million of dry soil after 83 days		
		19 days	55 days	83 days	Ammonia	Nitrate	Total
Arable Soil							
Untreated Soil	Colpoda, Amoebae and Monads	5·8	10·8	10·9	1·4	17·4	18·8
Soil + 0·1 % CaO	,, ,, ,,	8·8	11·1	10·9	1·4	19·3	20·7
Soil + 0·5 ,,	,, ,, ,,	10·9	40·9	64·5	15·0	18·0	33·0
Soil + 1·0 ,,	Amoebae and Monads	1·5	1·7	1·5	23·2	11·7	34·9
Garden Soil							
Untreated Soil	Colpoda, Amoebae and Monads	12·8	15·6	8·6	1·8	17·4	19·2
Soil + 0·1 % CaO	Hypotricha, Colpoda, Amoebae and Monads	14·3	31·3	11·0	2·0	28·8	30·8
Soil + 0·5 ,,	Amoebae and Monads	202·0	122·0	103·0	1·9	66·0	67·9
Soil + 1·0 ,,	,, ,,	99·2	454·6	498·6	40·6	21·3	61·9

The addition of 0·1 per cent. calcium oxide to the arable soil was without effect on the numbers of bacteria throughout the course of the experiment, and failed to induce any increase in the ammonia and nitrate nitrogen. Heavier applications of 0·5 per cent. oxide gave rise to increased bacterial growth within the first 55 days and this continued for at least 83 days, whilst 1·0 per cent. exerted an inhibitive

effect for the whole period. In spite of the low bacterial content in the latter case, there had occurred an accumulation of free ammonia thus indicating considerable chemical action of the lime itself.

As regards the garden soil, attention may be drawn to the transitory effect produced by small applications of lime. Here there occurred an initial rise of bacteria to upwards of 30 millions per gram and a subsequent drop to the normal content of the soil. Heavier doses would appear to be rendered innocuous to bacteria within the first 19 days and give rise to a phenomenal increase in the numbers of bacteria, which, particularly in the case of the soil with 1·0 per cent. oxide, is so much above that normally found in a soil treated with chemically inert volatile antiseptics.

These high bacterial numbers might possibly be due to direct chemical action, resulting in a liberation of simpler carbonaceous compounds, which alone would lead to increased bacterial growth. On the other hand, it might be attributed to the survival and subsequent increase of certain bacteria which are normally killed by treatment with toluene. It is often found that inoculation of a toluened soil with an extract containing the bacteria of an untreated soil, results in a much higher bacterial content, and a greater amount of chemical change, than is attained in the toluened soil alone, due no doubt to the growth of more active but less resistant species of bacteria.

Similarly, nitrifying organisms are always killed by toluene but would appear to remain alive in these limed soils in the majority of cases, or to find their way into the soil from the sides of the bottle, since it is impossible to sterilise it so efficiently by the use of lime as it is by means of heat or volatile antiseptics.

In any case, however, the death of protozoa causes a liberation of nitrogenous material not normally available and this in itself would lead to increased bacterial growth; that such a liberation does take place is shown by experiments carried out in the following manner: Portions of 500 grams of arable soil were mixed with calcium oxide in the proportion of 0·1, 0·25, 0·5 and 1·0 per cent. and thoroughly agitated with 500 c.c. of distilled water and allowed to stand at laboratory temperature for 24 hours. The soil suspension was then filtered, the filtrate being taken for titration and nitrogen estimations, whilst the residue was examined for bacteria after 6 and 22 days.

The amount of extractable nitrogen in this soil would appear to be in direct relation to the weights of lime used, in fact, the curve plotted from these data is quite linear in character. No decided change

in the reaction of the filtrate occurs except in the case of the heaviest dressing of lime, although the slight change from an acid to an alkaline reaction of the filtrate is also accompanied by higher bacterial numbers after a period of incubation.

TABLE III. *Showing the liberation of soluble Nitrogen Compounds from the Soil by Caustic Lime.*

	Control	Percentage of Calcium Oxide added to Soil			
		0·1 %	0·25 %	0·5 %	1·0 %
Soluble nitrogen in filtrate (excluding nitrates)	1·52 mgrm.	3·80 mgrm.	5·32 mgrm.	8·36 mgrm.	9·88 mgrm.
Titration of filtrate 25 c.c.	=0·05 c.c. $\frac{N}{10}$ NaOH	=0·05 c.c. $\frac{N}{10}$ NaOH	=0·05 c.c. $\frac{N}{10}$ H_2SO_4	=0·1 c.c. $\frac{N}{10}$ H_2SO_4	=4·2 c.c. $\frac{N}{10}$ H_2SO_4
Protozoa	Colpoda, Amoebae, Monads	Colpoda, Monads	Colpoda, Monads	Monads	Monads
Bacteria (in millions) per gram of soil after					
6 days	15·8	30·5	29·7	31·7	19·6
22 days	22·3	32·1	69·8	54·2	68·2

The bacterial counts in all the treated soils show a uniform rise after six days, with the exception of the sample receiving 1·0 per cent. of lime in which recovery from an initial depression would appear to be just taking place. After 22 days the bacteria in the soil receiving 0·1 per cent. have scarcely increased, whilst those in the other three samples have multiplied considerably.

As regards the bacterial growth, therefore, more favourable conditions occur with the addition of 0·25 per cent. than with 0·1 per cent. oxide, but whether this is a permanent increase or merely a temporary rise to be followed by a fall in numbers, such as is often met with in soils incompletely freed from the larger protozoa, is a point that can only be decided by further tests.

Somewhat similar experiments have been carried out with an acid soil from the Woburn Experimental Station[1], and also a rich soil (containing about 1·0 per cent. calcium carbonate) from the Chelsea Physic Gardens. In this case 100 grams of soil were mixed with various quantities of oxide and 50 c.c. of distilled water, and after

[1] This soil was obtained from one of the Woburn plots which have been rendered distinctly acid by continuous applications of ammonium sulphate, and was kindly placed at the disposal of the author by Dr J. A. Voelcker, Director of the Station.

shaking, the samples were allowed to stand for 4 hours and then filtered. The residue was washed with a further quantity of 50 c.c. of water, and the whole of the filtrate taken for nitrogen estimations.

TABLE IV. *Showing the action of Caustic Lime on the liberation of soluble Nitrogen Compounds and on Bacterial growth.*

	Woburn Soil		Chelsea Soil	
	Nitrogen in 100 c.c. of filtrate	Bacteria (in millions per gram of soil) after 3 days	Nitrogen in 100 c.c. of filtrate	Bacteria (in millions per gram of soil) after 3 days
Control	0·95	9·7	0·55	22·2
0·1 % CaO	0·80	16·5	—	27·0
0·25 ,,	1·45	71·4	0·90	23·9
0·5 ,,	2·50	6·2	3·45	13·9
1·0 ,,	2·70	2·5	2·35	3·9

The washings of the Woburn soil resemble, with respect to soluble nitrogen, those obtained from the Rothamsted arable soil.

The effect of supplying calcium oxide in doses of 0·25 and 0·5 per cent. is to increase markedly the amount of extractable nitrogen and this is not appreciably raised by using double the amount of lime. On the other hand, the greater part of the lime in the case of the Woburn soil receiving 0·25 per cent. would appear to have entered into combination or to be extracted by the wash-water employed, thus leaving the soil in a favourable condition for bacterial growth and resulting in the relatively high content of 71·4 millions per gram. Heavier applications reduce bacterial numbers greatly and would doubtless continue to exercise this effect for some considerable period in the case of such a light soil as the one under consideration.

Comparable results were obtained with the Chelsea soil. On account of the presence of a sufficiency of calcium carbonate in the soil, the addition of lime up to 0·25 per cent. was without any decided effect either on the amounts of soluble nitrogen or bacteria, but greater doses lead to increased liberation of nitrogen and decreased initial bacterial numbers.

Pot Experiments.

Since the foregoing results appeared to indicate an action of caustic lime which had not previously been recognised, it was considered

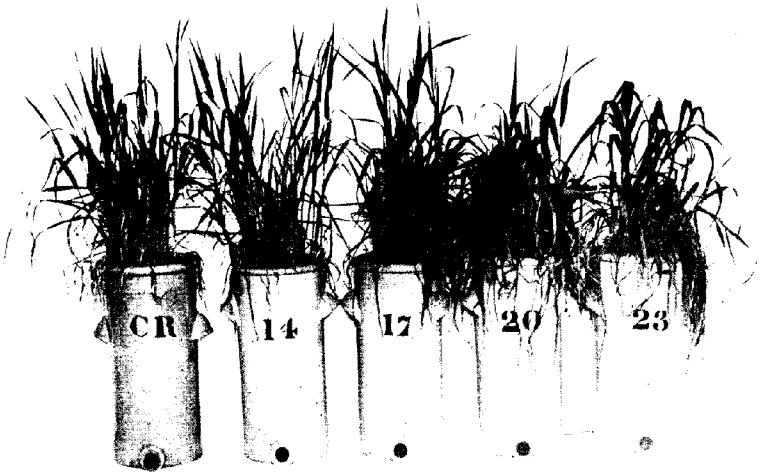

Fig. 1.
First Crop: Barley.

Fig. 2.
Second Crop: Mustard.

Experiments on the action of Caustic Lime on Garden Soil. Treatment: C.R. untreated soil. Nos. 14, 17, 20, and 23 soil treated with 0·9 per cent. calcium carbonate, and 0·1, 0·5 and 1·0 per cent. calcium oxide respectively.

desirable to carry out similar experiments in pot culture with the same arable and garden soils as were used in earlier laboratory tests. Glazed pots containing 18 lbs. and 20 lbs. of arable and garden soils respectively were used, and lime was added in the proportion of 0·1 per cent., 0·5 per cent. and 1·0 per cent. With each of the soils a series of pots was included where an addition of calcium carbonate equivalent to 0·5 per cent. of the oxide was made, in order to show that the results obtained were not due to any increase of carbonate in the soil, but rather to a specific action of the lime in the caustic state.

The mixtures of soil and lime were allowed to stand for three weeks under cover, at the end of which time they were turned out, the reaction of the soil tested with litmus paper, and wherever distinctly alkaline, carbon dioxide was passed through. Only in one case, namely, that of the poor arable soil with 1·0 per cent. oxide, was the soil alkaline and a strong smell of ammonia was to be detected.

Ten barley seeds were sown in each pot on May 2, 1912, and germination was regular in all soils except in those which had received 1·0 per cent. oxide, where a slight but noticeable retardation was apparent; this effect persisted for some considerable time especially in the case of the arable soil.

On the whole the addition of caustic lime caused growth entirely characteristic of plants growing in partially sterilised soils: the plants were stunted and of a dark bluish green colour. The crop was cut on August 14th, and the average weights of dry produce per set of three pots are given in Table V. After the barley roots had been taken out a fresh crop of mustard was sown on August 21st, and showed greatly increased growth in those soils which had received lime in quantities sufficient to induce partial sterilisation effects in laboratory experiments. Photographs of the crops in garden soils are shown on Plate XIV.

It is difficult to account satisfactorily for the uniform depression shown by the first crop in garden soils with each addition of caustic lime. Bacteriological analyses of these soils, made before the barley was sown, showed that an appreciable increase in the numbers of bacteria had already taken place, and thus indicated that much if not all the oxide applied had been converted to the mild form. Furthermore, in the case of the arable soil there was no such depression with the two applications of 0·1 and 0·5 per cent. of oxide; decreased growth in the soil with 1·0 per cent. of oxide is not surprising when one bears

in mind the persistence of caustic lime in this soil, as shown by earlier bacteriological and chemical analyses.

It is proposed to extend these studies to various types of soils with a view to ascertaining, if possible, what applications of lime are necessary to induce partial sterilisation in each case, the rapidity of conversion of the lime from the caustic to the mild form, and the nature of the injurious action on crops where such is evident. In the consideration of these results and their relation to field conditions it should be remarked that in converting percentages of lime into tons per acre, an addition of 0·1 per cent. to a soil is almost equivalent to a dressing of one ton per acre calculated on the top nine inches of soil. Since, however, the effective layer, as far as numbers of bacteria are concerned, is constituted by the top six inches, the quantities used in these experiments would be about 0·6, 1·6, 3·3 and 6·6 tons per acre. The latter dressings would certainly appear to be in excess of those generally advised and adopted in farm practice, but this does not necessarily mean that they are too high, whilst the scarcity of data showing the effect of various quantities of caustic lime on arable land precludes any attempt at correlation.

On the other hand, the amounts applied about the middle of last century varied from 3—10 tons per acre, according to the state and character of the soil, in order to produce maximum results. Were these maximum results due, in any degree, to partial sterilisation of the soil?

TABLE V. *Showing the Results of pot experiments with Untreated and Limed Soils.*

Treatment	Arable Soil			
	Dry produce per pot (average yield of three pots)			
	1st crop, Barley		2nd crop, Mustard	
	in grams	relative weight	in grams	relative weight
Untreated Soil	9·5	100	0·55	100
Soil + 0·9 % $CaCO_3$	11·1	117	0·55	100
Soil + 0·1 % CaO	12·4	130	0·50	91
Soil + 0·5 „	15·4	162	1·16	211
Soil + 1·0 „	3·8	40	3·88	705

Treatment	Garden Soil			
	Dry produce per pot (average yield of three pots)			
	1st crop, Barley		2nd crop, Mustard	
	in grams	relative weight	in grams	relative weight
Untreated Soil	28·6	100	2·28	100
Soil + 0·9 % $CaCO_3$	28·4	99	1·86	81
Soil + 0·1 % CaO	24·7	86	1·92	84
Soil + 0·5 „	24·5	86	5·51	242
Soil + 1·0 „	24·6	86	9·50	417

Conclusions.

When a soil is treated with lime, either in the caustic or mild form, an improvement of its physical condition results; the treatment gives rise to a certain amount of chemical action with a liberation of nutrient substances, and also, by neutralising any acids present, provides a more favourable environment for the various classes of organisms existing in the soil.

This in itself is not sufficient to account for many of the results that are obtained in practice. Caustic lime has a recognised value as an antiseptic and, when applied to the soil, even in the presence of large quantities of calcium carbonate, has a pronounced effect in disturbing or even destroying the state of equilibrium normally existing between the micro-flora and the micro-fauna of the soil.

The action of caustic lime has been found to be intermediate in character between that exercised by volatile antiseptics and the changes induced by high temperatures. In addition to killing many bacteria and causing the death of the larger protozoa, which would appear to exert a depressive action on the growth of bacteria, it brings about a decomposition of the organic nitrogenous constituents of the soil. It is highly probable that these decomposition products serve as nutrients for bacteria and are subsequently resolved into plant food.

The depression of bacterial activities in soils treated with caustic lime would appear to persist until all the oxide has been converted into carbonate; this is followed by a period of active bacterial growth and

increased production of plant food. The inhibitory action of caustic lime on soil bacteria varies with the soil and is possibly governed by the organic matter present.

In the main, pot experiments give results similar to those obtained in the laboratory by bacteriological and chemical analyses. A poor arable soil, containing a sufficiency of calcium carbonate, gave increased yields when treated with 0·5 per cent. of calcium oxide. A rich garden soil, on the other hand, gave decreased yields in the first crop but largely increased yields in the second crop. The conditions leading to this depression are not clear at present and confirmatory experiments are necessary before any explanation can be attempted. It is, perhaps, worthy of note that similar depression is often observed with soils that have been subjected to high temperatures.

OBSERVATIONS ON THE FAT GLOBULES IN MILK.

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Introduction.

It is a well-known fact that creams vary in the process of churning; it is known that some creams take longer than others to "come"; it is known also—and this is of far greater importance—that the butter obtained varies very greatly, both in quantity and quality. But the reasons for these variations are not known. The question arose, some time ago, whether any function of the globules of fat (size, number, etc.) influenced this variation.

This point was taken up by us, at the request of Mr Ernest Mathews, in 1909, and has formed the subject of much work in the laboratory at Watford.

That the globules do have an effect upon the churnability is shown by several writers. For instance, Sturtevant (*Ann. Report, U.S.D.A.*, 1880; Abridged Account in *Journ. Royal Agric. Soc. Eng.*, 1882, xviii, (2), 475—495) showed that "the larger the globule, other things being equal, the quicker the churning." He states that a milk with larger globules gives better butter, and also that "churning is a physical process and acts upon the larger globules only."

It has been stated by Klusemann (*Inaugural Dissertation*, Leipzig, 1893; Abst. in *Chem-Zentralblatt*, 1894, (1), 646; *U.S.D.A. Expt. Stat. Record*, v, 1022) that butter made from milk with large globules has a deep yellow colour, a good taste, and is hard; whereas that made from a milk with small globules has a whitish colour, and is inferior in taste and consistency. He concludes also that the greater the proportion of large globules, the more completely can the cream be churned. But no information is given as to whether this observation is made upon milk

from cows of the same breed; for it is very well known that the breed has a great influence on the colour of the butter.

At the outset, the problem did not appear to be very difficult; but as the work progressed, it was obvious not only that the experimental work was by no means simple, but also that the results were very inconclusive. At first, consideration was given to the globules solely; but it was soon realised that a large number of other factors affected the problem. Moreover, it was quite impossible to give consideration to one variant only, so that it was difficult to determine to what factor any particular result might be due.

Of the variable factors which might affect "churnability[1]," the following are the chief:—

(*a*) The physical properties of the milk.
(*b*) The size, number and distribution of the globules.
(*c*) The condition of the serum immediately surrounding the globules—a covering or membrane.
(*d*) The effect of food, whether on the globules or on the serum.
(*e*) The constitution of the serum.
(*f*) The temperature of the cream.
(*g*) The ripeness of the cream.
(*h*) The percentage of fat in the cream.
(*k*) The breed of cow.
(*l*) Period of lactation.
(*m*) The influence of bacteria.

Before it is possible to determine whether any variation is due to a function of the globules themselves, it is necessary to ascertain the general effect of the other factors.

The size, number and distribution of the globules have been considered in a previous paper (*Journ. Agric. Sci.*, IV, 1911, Pt. 2, 150—176); further results are given below (Tables V—XIII, Figs. 3—6), and a résumé is given of the present knowledge of the much debated problem of the membrane surrounding the globule. The effect of the feed upon the globules forms the main portion of this communication, but its effect on the serum has not been investigated. The constitution of the serum, the effect of the temperature of the cream and the percentage of fat in the cream, upon the churnability, have been considered and will be discussed later. Attention has not yet been given to other factors.

[1] The term "churnability" is used in a general sense to include all the variations which occur in churning.

SUMMARY OF PREVIOUS WORK.

Results of our previous work were published in the *Journ. of Agric. Sci.*, IV, 1911. There, also, a brief summary of the chief papers published on the subject of the fat globules was given, to which it is unnecessary to refer in detail here[1].

When the work was first commenced in 1909, the problem before us was the consideration of the variation in the size of the fat globules, with relation to churning, as regards the *different breeds* of cattle. The breed was considered because it was usually supposed that this was one of the chief factors which influenced churning. The most definite result of our work was that it was shown that consideration must be given to *the character of the milk, irrespective of the breed.* This conclusion is quite contrary to that of other workers, but an examination of their figures shows, undoubtedly, that this is actually the case: the results given by Woll (Digestion Expts., *Seventh Annual Report, Agric. Expt. Stat., Wisconsin*, 1890, 238; also *Agric. Sci.*, 1892, VI, 445) emphasise this point particularly. It is also shown by this year's work, as may be seen from Tables V—XI.

The comparative size of the globules has been worked out very thoroughly by Gutzeit, who measured the mean volume of the globule. Other workers give their results as "relative sizes." In our work we attempted to ascertain the distribution of the fat in the globules, and to this end the number of globules of each size was determined, and curves were drawn. The result of this, however, was negative. At that time we were considering the *breeds of the cows* from which the milks were obtained; had we considered them solely as milks of a certain mean size of globule, much more might have been achieved.

An apparatus was devised to give an absolute figure for the churnability of any cream, but, until the effect of the other factors has been determined, it is impossible to interpret the results. Some experiments to ascertain the optimum temperature were described also.

CONSTITUTION OF THE SERUM.

In addition to the globules themselves, one must consider the causes which prevent them from coalescing. This may be due to

[1] In the English literature, where the subject of globules is dealt with, reference is given chiefly to D'Hont's paper ("Essai sur les dimensions du globule gras en suspension dans le lait. Influence de la race." Courtrai, 1890). This is remarkable since a work has been published by Gutzeit (*Landwirtsch. Jahrbücher*, XXIV. Berlin, 1895, 539–668) which is very exhaustive, and which is based on a very large number of actual measurements.

some quality of the serum in which they are floating. For instance, Babcock (*New York Expt. Stat., Fifth Report,* 1887) investigated the viscosity of various milks, and found that the viscosity of milk is greatest with a large mean-size globule. Of the constituents of milk serum, the albuminoids have by far the greatest influence on the viscosity. He states that "a low coefficient of viscosity for the serum is most favourable to the economical production of butter." On the other hand, coalescence may be prevented by some covering round the globules. This point is considered later.

In the work of 1910, published in the *Journ. of Agric. Sci.*, analyses were made to determine whether any correlation existed between the proteids and sugar in the serum, and the fat and the size of globule in the milk. No definite conclusion can, however, be drawn.

Hart (*Journ. Amer. Chem. Soc.*, XXX, No. 2, 1908, 281—285), who investigated this point, showed that:—

(1) The relation of casein to fat is variable.

(2) It is due, chiefly, to individuality of the cows.

Brevans (*Hyg. Viande et Lait*, III, 1909, No. 12, 593) also gave tables to show the effect of individuality.

In 1911, therefore, consideration was given to the nitrogen-containing constituents. Analyses were made upon those milks, the creams of which were used for the churnability and for optimum temperature experiments. The total nitrogen was determined by Kjeldahl's method; the total proteids by precipitation with tannic acid; the casein by precipitation with magnesium sulphate; the albumen by difference. The results are given in Table I.

TABLE I.

	Jersey		Guernsey		Kerry		Red Poll	
	Whole milk	Cream	Whole milk	Cream	Whole milk	Cream	Whole milk	Cream
Per cent. fat	Broken in transit	58·21	4·10	57·91	3·92	57·08	3·97	57·91
,, N. × 6·38		1·50	3·34	1·42	3·29	1·53	3·31	1·57
,, non-proteid N.		0·22	0·22	0·01	0·28	0·11	0·18	—
,, total proteids		1·28	3·12	1·41	3·01	1·44	3·13	1·56
,, casein		1·14	2·37	1·17	2·49	1·105	2·62	0·88
,, albuminoids (?)		0·14	0·75	0·24	0·52	0·32	0·51	—
$\frac{\text{Total proteids}}{\text{Total N.} \times 6{\cdot}38} \times 100$		85·3	93·4	99·4	91·6	93·7	94·4	99·6
$\frac{\text{Casein}}{\text{Total proteids}} \times 100$		89·4	76·1	83·1	82·6	76·8	83·9	56·5

No correlation could be found between these figures and those obtained with the churnability apparatus and the other determinations which were made; this is probably due to the large number of factors involved.

Optimum Temperature.

It is known that better results are obtained by churning at a low temperature; but, when the literature was examined in order to see upon what experiments this knowledge was based, it was impossible to find any *definite* data.

Some excellent tests were carried out at the various shows of the Royal Agricultural Society, but no precautions were taken to keep the temperature constant. Indeed, on examining some of the results—excellent *practical* results—one finds that the temperature varied, in some cases, between 54° F. and 60° F.; that is to say, practically over the whole range of churning temperature. These were *practical* tests, and it is difficult to see how they could have been carried out differently, without entirely modifying the scale and the method.

In this connection, Robertson (*Canada Expt. Farms Report*, 1892, 71—78; Abst. in *U.S.D.A. Stat. Record*, v, 1894, 641) carried out some experiments on sweet cream at temperatures ranging from 41° F. to 58° F. at the *beginning* of churning. The temperature at the *conclusion* of churning was from 57° F. to 62° F., so that here also no precautions were taken to keep the temperature constant. His results showed that:—

(1) With an initial temperature of 50° F., or under, the quantity of the fat remaining in the butter-milk need not exceed 0·25 %.

(2) For the efficient conversion of the fat, the temperature of the cream should not be above 50° F. *at the commencement.*

(3) That the churn (if a revolving one) should not be more than one quarter full.

Robertson made experiments also on the addition of water to the cream, and showed that there was slightly less percentage-conversion where water had been added to the cream before ripening. He showed, moreover, that the period of lactation had an influence on the conversion, inasmuch as the loss of fat was greatest with the milk of cows which had been more than six-and-a-half months in milk.

In 1909 an attempt was made to determine the optimum temperature definitely, and the apparatus and method were described in detail in the *Journ. Agric. Sci.*, iv, Pt. 2, 1909, 167. It was found that

at higher temperatures the percentage of the fat taken in the cream, which was converted into butter, varied.

The work was repeated in 1911, and in this case not only the temperature but also the percentage of fat in the cream was varied. It seemed to be desirable that the percentage of fat in the cream should be as near as possible to that taken in practical work. Seven samples of cream were taken, as prepared ready for churning by seven different dairy-maids. The fat was found to vary from 36°/₀ to 45·5°/₀, the average being 38·5°/₀. A cream containing 38°/₀ fat would not churn in our apparatus, however; 30°/₀ was the maximum, and even in that case it was necessary to add "breaking" water. Experiments were made, therefore, using fresh cream containing 30°/₀ and 25°/₀ of fat respectively. 200 grams were taken of each cream, and these were churned at 54° F., 58° F. and 62° F. Where "breaking" water was added towards the end of the churning, the amount was noted. The butter-milk was weighed, and the percentage of fat in it was determined; from this the percentage of fat lost in the butter-milk was calculated.

The results are shown in Tables II and III, and are plotted in curves in Figures 1 and 2.

These results were obtained from one series of experiments only; for at that time the various breeds were being considered, and these could only be obtained at the Royal Show, where it was impossible to make duplicate experiments. Now that it is realised that the milks should be considered in respect to the size of their globules, rather than to the breed, it is possible to repeat the experiments in the laboratory. This it is proposed to do in the near future.

The most definite conclusion which can be drawn from these experiments is that the percentage of fat in the cream has a very marked influence upon the percentage conversion of fat into butter.

It is obvious that the temperature has a considerable influence, but to what extent, these results can only afford an approximation.

The effect of temperature is not marked in the case of Jersey, Guernsey or Kerry milks, provided that the cream is thick. In the other breeds, the effect is more pronounced; but in these cases better results might have been obtained if the cream had contained more fat. In all cases, however, the effect of temperature is very marked if the cream is too thin.

In the tables opposite, the names of the breeds are given, but it must be remembered that the results, as shown, are not necessarily any criterion as to the suitability of any particular breed for dairy work. This point is considered more fully on p. 356.

Table II.

Breed	Mean Dia.	Temp. Deg. F.	% Fat	Cream taken gms.	Fat taken gms.	Water added gms.	Weight of Butter obtained gms.	Weight of Butter-milk gms.	% Fat in Butter-milk	Weight of Fat in Butter-milk gms.	% Fat lost
Shorthorn		54				70	68	202	0·75	1·5	2·6
		58	29	200	58	—	66·2	133·8	2·3	3·1	5·3
		62				—	63·8	136·2	4·4	6·0	10·3
Jersey		54				130	72·5	257·5	0·1	0·25	0·4
		58	30	200	60	100	72·0	228·0	0·2	0·45	0·75
		62				70	78·7	191·3	0·3	0·6	1·0
Guernsey	3·09	54				70	68·8	201·2	0·2	0·4	0·7
		58	30	200	60	70	69·4	200·6	0·2	0·4	0·7
		62				—	72·0	128·0	0·45	0·6	1·0
Red Poll	2·84	54				—	70·0	130·0	2·25	2·9	6·0
		58	30·5	200	61	—	69·0	131·0	3·8	4·9	8·0
		62				70	70·1	199·9	5·58	11·1	18·2
Kerry	2·88	54				70	73·2	196·8	0·1	0·2	0·3
		58	30	200	60	70	68·8	201·2	0·4	0·8	1·3
		62				70	70·2	199·8	0·5	1·0	1·6
Ayrshire		54				70	74·2	195·8	0·5	1·0	1·6
		58	30·5	200	61	70	77·2	192·8	0·75	1·4	2·3
		62				—	75·6	124·4	2·6	3·2	5·2

Table III

Breed	Mean Dia.	Temp. Deg. F.	% Fat	Cream taken gms.	Fat taken gms.	Water added gms.	Weight of Butter obtained gms.	Weight of Butter-milk gms.	% Fat in Butter-milk	Weight of Fat in Butter-milk gms.	% Fat lost
Shorthorn		54				—	61·5	138·5	2·2	3·0	6·0
		58	25	200	50	—	41·7	158·3	10·3	16·3	32·6
		62				—	49·5	150·5	9·9	14·9	29·8
Jersey		54				50	64·8	185·2	0·4	0·7	1·4
		58	25	200	50	—	51·7	148·3	5·4	8·0	16·0
		62				—	44·2	155·8	6·9	10·7	21·4
Guernsey		54				—	66·0	134·0	1·2	1·6	3·0
		58	26·75	200	53·5	—	51·7	148·3	7·7	11·4	21·3
		62				—	43·7	156·3	8·1	12·6	23·5
Red Poll		54				—	63·7	136·3	3·9	5·3	10·0
		58	26·75	200	53·5	—	59·1	140·9	10·1	14·2	26·5
		62				—	55·6	144·4	10·8	15·6	29·2
Kerry		54				—	61·5	138·5	1·2	1·6	3·2
		58	25	200	50	—	52·3	147·5	2·7	4·0	8·0
		62				—	48·5	151·5	9·9	15·0	30·0

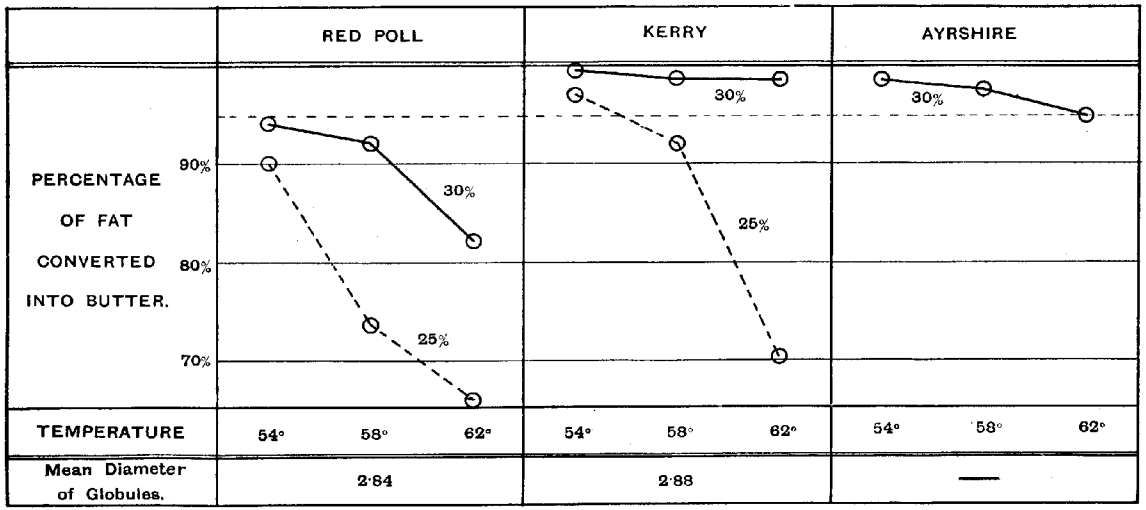

Fig. 1. Curves showing effects of different temperatures on the percentage conversion of milk fat into butter.

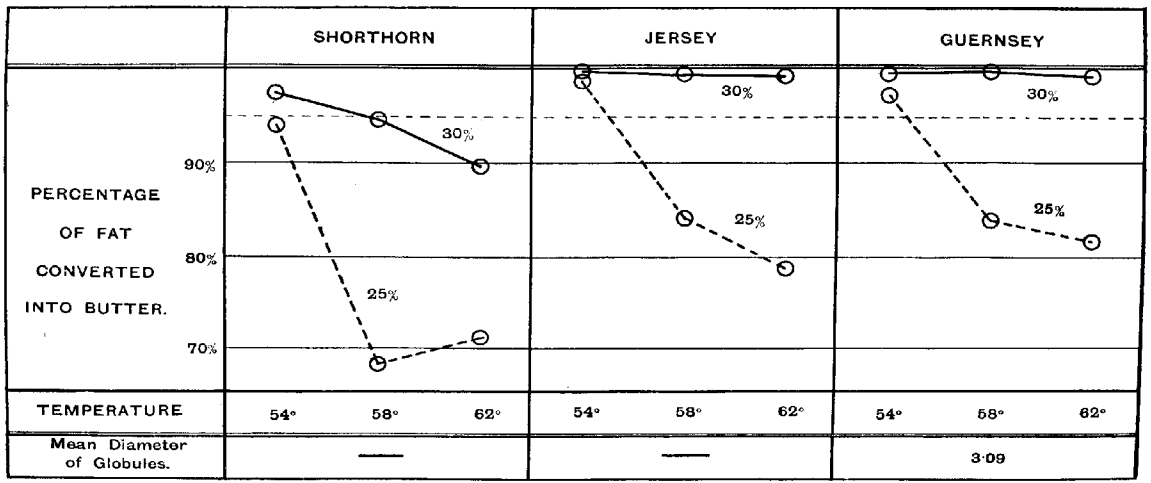

Fig. 2. Curves showing effects of different temperatures on the percentage conversion of milk fat into butter.

For reference, the mean size of the fat globules contained in the milks from which the creams were obtained is given below three of the curves.[1] No correlation could be determined between the percentage conversion and the size of globule.

That the size of globules has an effect on the percentage conversion, however, is shown by Woll (Digestion Expts., *Seventh Ann. Report, Agric. Expt. Stat., Wisconsin*, 1890, 238; also *Agricultural Science*, VI, 1892, 545), who concluded that "large size in the fat globules facilitates both the creaming and the churning of the milk; but the size of the globule is only one of the factors influencing completeness of creaming in churning processes." By mixing milks containing globules of different sizes "the creaming efficiency does not seem to be materially affected."

The Limiting-Surface of the Globules.

In problems connected with churnability, a knowledge as to the presence or absence of a covering to the globules is important.

A very brief account of the different views put forward may be of interest. The various opinions may be classified in three categories. There are those who hold that there is an actual skin, membrane or pellicle round the globule. There are those who consider that there is nothing more than a "sliminess" round the globules; a condition which appears to us to be described best as a *mucilaginous aggregation*. Finally, others deny the existence of any covering whatever.

The membrane theory was first put forward by Raspail (*Schmidt's Jahrbuch*, XXIV) and by Acherson (*Müller's Arch. f. Anat. u. Physiol.*, LIII, 1840), so that the pellicle is often termed Acherson's membrane, though Acherson himself suggested the term *haptogen membrane*. Voltz (*Pflüger's Arch. f. Physiol.*, CII, 1904, 373) carried out a lengthy and detailed research, and concluded that "the fat globules of milk possess pellicles of solid substances, probably actually *solid membranes*."

Of the second view, perhaps the best exposition is that of Storch ("On the structure of the 'Fat Globules' in Cow's Milk," *Analyst*, XXII, 1897, 197—211), who isolated an albuminous substance which he claimed was the actual membrane. He expressly states, however, that "by the term membrane, is not meant a solid film or skin, but merely a layer, a semi-fluid, viscous envelope,"—evidently a "sliminess" round the globules. The use of the terms "membrane," "skin," "envelope," etc.,

[1] The other figures cannot be given.

appears to be unfortunate for the description of such a condition; it causes a misconception, as is evident from some of the criticisms of Storch's work. For this reason, together with one to be mentioned later, we propose the term *mucilaginous aggregation.*

With regard to the substance isolated by Storch, Voltz (*Pflüger's Arch. f. Physiol.*, CII, 1904, 373) more recently has obtained a substance which surrounded the globules even after they had been very thoroughly washed, but he found that there were extraordinary variations in the composition, according to the conditions of the experiment. Both these workers were able to stain the substance round the globule, but each drew different conclusions.

In our opinion, the divergent results obtained and the views held by the different workers are all to be reconciled on the theory that *constituents of the milk serum are adsorbed to the surface of the globule.*

On this theory, milk would not differ essentially from many artificial emulsions. It is generally accepted that milk differs from an ordinary emulsion in that the fat is not extractable with ether. If sufficient ether is used, however, the fat can be removed completely. Further, a number of artificial emulsions—*cyllin* in water, for example—behave exactly like milk in this respect, the oil being removed by ether only with great difficulty. In both cases the fat is comparatively easily extracted if the emulsifying agent is broken down. Moreover, it is possible to detect and to isolate the aggregated adsorbed emulsifying agent surrounding the globules in an artificial emulsion, so that it is only reasonable to conclude that similar conditions prevail in milk. In short, the difference between milk and an artificial emulsion appears to be one of degree only, and that difference is to be explained by the phenomenon of adsorption.

In this connection, the paper of Marshall (*Pharm. Journ. and Pharmacist*, Feb. 27th, 1909) and that of Ramsden ("Observations on Surface Membranes, Bubbles, Emulsions, and Mechanical Coagulation," *Chem. News*, LXXXVIII, 1903, 49—51; also *Proc. Royal Soc.*, LXXII, 1904, 458) are of considerable interest.

An actual solid membrane is not incompatible with the theory, as is clearly shown by Ramsden (1903) when discussing this theory:—"An intense *special interface viscosity*...has been found with every pair of liquids capable of forming persistent emulsions, hitherto examined." And "...the presence of a pellicle at the interfaces between caseinogen solutions and pure butter fat...." Also "the existence of a proteid

'Haptogen-membrane' round the cream globules of milk cannot be any longer doubted."

Storch's conclusions are based upon the result of one experiment. Voltz made repeated experiments, and obtained substances which varied greatly in composition according to the conditions. An examination of his figures shows that it is what one would expect, according to the adsorption theory, but they have not been submitted to mathematical analysis, since the data are insufficient.

Feeding Experiments—1912.

Several observers have noted that the food has an effect upon the globules, but, unfortunately, their results are contradictory.

Sturtevant (*Ann. Report U.S.D.A.*, 1880; Abridged Account in *Journ. Royal Agric. Soc. Eng.*, XVIII, (2), 1882, 475—495) concluded that *brans* and *shorts* diminished the size of the globule, whereas corn-meal "influenced uniformity." Woll (*Sixth Ann. Report, Agric. Expt. Stat., Wisconsin*, 1889, 122; also *Seventh ditto*, 1890, 246) stated that dry food decreased the number and increased the size of the globules. Corn gave the same result as dry food. Gutzeit (*Landwirtsch. Jahrbücher*, Berlin, XXIV, 1895, 539—668), who carried out an extremely large number of observations, came to the conclusion that it was *not* true that one food would increase the diameter and another diminish it; but that, with a sudden change of fodder, there was a temporary change in the size.

It should be observed that the differences found by Woll were small, and, according to our experience, are within the limits of experimental error. Moreover, they are expressed in "relative sizes," and not in mean diameters, as we have done; nor in mean volumes, as Gutzeit has done; so that the figures appear, at first sight, to be more conclusive than one might otherwise suppose.

As the matter is of great importance in the present work, it was considered advisable to carry out further experiments, not with any idea of ascertaining the influence upon the quantity and quality of milk, but solely with respect to the globules.

It was decided to take three sets of cows; to one, to give an ordinary food such as would be given normally, having a proteid-carbohydrate ratio of about 1 : 6; to another set of cows to give food having an abnormally high, and to the third set a food having an abnormally low ratio. The object of these abnormal foods was to

accentuate any difference, and it was considered advisable to accentuate them as much as possible, because there is a considerable difference of opinion as to whether the size of the globules is actually altered. Not only that, but there is a natural diminution in size as the period of lactation advances, so that, in any case, a diminution in size must be expected with those cows fed on the normal food; an effect which it was desirable to exclude as far as possible.

The experiments were carried out in a dairy very near to the laboratory, where some forty cows are milked. The breed is not pure, but is chiefly Shorthorn. They were being stall-fed, so that one could know exactly the quantity of food they received. Six cows were taken and were divided into three lots of two each, one lot for each ration.

In addition, Mrs Dudley Cory-Wright, of Dorking, very kindly allowed us to carry out similar experiments with three of her cows, two Guernseys and one Jersey. These received the same dry rations, but they were turned out, and therefore had an unknown quantity of grass, so that it is impossible to give ratios.

Photographs of the milk and other data were taken before the experiments commenced; then rations were given according to Table IV. Samples of the morning and evening milk were taken every third day. After 22 days[1], a measurement of the globules showed no alteration, and it was decided to modify the rations, so as to accentuate the ratios to an even greater extent.

Determinations were made every third day, as before, for 26 days, and then the food of the cows was changed over; so that those cows which had been having the food with a high ratio were given the food with the low ratio, and vice versa. The cows receiving the food with the normal ratio continued as before.

FOOD.

A decision as to the most suitable rations was very difficult, since discrepant figures are given for the composition of each material. As the chief authority, the figures of Crowther were taken. The rations given were based chiefly upon suggestions of Dr Hedworth Foulkes, and are tabulated below.

As to the materials, the grass was ordinary meadow grass, containing no clover. The hay was similar. The maize meal was ordinary material

[1] Woll (*Sixth Ann. Report, Agric. Expt. Stat., Wisconsin*, 1889, 71) also fed his cows 3 or 4 weeks, before changing the fodder.

purchased in Watford. The soya cake and cotton cake were obtained from Messrs Bibby, who very kindly made a reduction in price for the experiments, and who have been very obliging in many details. All the cake supplied was prepared specially in one batch, so that there should be no variation.

The rations shown in column A in Table IV were given for 22 days; after that the rations were given as shown in column B, having the corresponding ratios. The final examination was made on the 38th day. The cows were milked at 4.0 a.m. and at 1.0 p.m.

TABLE IV.

Rations (per cow per day)	High Albuminoid Ratio		Medium Albuminoid Ratio		Low Albuminoid Ratio	
	A. For 22 days	B. After 22 days	A. For 22 days	B. After 22 days	A. For 22 days	B. After 22 days
Meadow grass (no clover)	42 lbs.	42 lbs.	56 lbs.	56 lbs.	42 lbs.	42 lbs.
Hay	—	—	—	—	14 lbs.	14 lbs.
Soya cake	4 lbs.	6 lbs.	—	—	—	—
Cotton cake (undec.)	—	—	3 lbs.	5 lbs.	—	—
Maize meal	—	—	—	—	4 lbs.	6 lbs.
Digestible Food Ratio*	1 : 2·75 : 0·24	1 : 2·2 : 0·22	1 : 5·2 : 0·33	1 : 4·4 : 0·33	1 : 8·9 : 0·36	1 : 8·9 : 0
Total solids	12 lbs.	13·75 lbs.	14 lbs.	15·5 lbs.	24 lbs.	25·75 lb
Quantities of Constituents per Ration						
Total albuminoids	3·0 lbs.	3·8 lbs.	2·3 lbs.	2·9 lbs.	3·1 lbs.	3·3 lb
,, carbohydrates	5·3 ,,	5·8 ,,	6·6 ,,	7·3 ,,	13·0 ,,	13·3
,, fat	0·6 ,,	7·1 ,,	0·6 ,,	0·7 ,,	0·8 ,,	1·0
Digestible albuminoids	2·0 ,,	2·7 ,,	1·3 ,,	1·6 ,,	1·5 ,,	1·6
,, carbohydrates	5·5 ,,	5·9 ,,	6·8 ,,	7·2 ,,	13·1 ,,	13·4
,, fat	0·5 ,,	0·6 ,,	0·4 ,,	0·5 ,,	0·5 ,,	0·6

* These ratios are arranged :—*albuminoid, carbohydrate, fat.*

The method of mensuration of the globules has been given in a former paper (*Journ. Agric. Sci.*, IV, Pt. 2, 1911, 155—166) but a detailed description, together with an account of Babcock's method, appears in a subsequent portion of this issue[1], p. 357.

[1] It would be well to emphasise, once more, the futility of attempting to judge either of the average size of the globules or of the fat content in a milk, by visual examination. It must not be supposed that a field full of small globules contains more fat than one with a small number of large globules; or vice versa; determination alone can decide this.

It should be pointed out that, in Babcock's method, it is not possible to check the results. In our method, however, this can be done as pointed out below; any discrepancy may be due to erroneous counting, to bad sampling, or to selection of a bad field for the photograph, and the count can be neglected as being erroneous.

The two methods of computation of the mean diameter are[1]:—

A. The globules can be counted and the size of each measured, so that the distribution of the fat in the different sizes of globules can be determined. From the numbers so obtained curves can be drawn which show graphically the distribution of the fat.

B. Where the mean diameter alone is required, the number of globules in a known area is counted; the depth of the cell is known, so that the volume of milk in the area measured is known also; the percentage of fat, determined by analysis[2], is reduced to give the volume of fat in the volume measured; this fat is distributed in the known number of globules, so that the mean diameter of the globules can be calculated. This is the method by which the mean diameters have been calculated for Tables VI—XIII and Figs. 3—6.

For some of the milks the mean diameters have been calculated also by method A, and the figures thus obtained are bracketed in Tables VI—X. The agreement or disagreement of these two figures is a measure of correctness of the determination, and an asterisk is placed against those in which there is an appreciable discrepancy.

An excellent instance of the value of this dual method of calculation is afforded by the following. The milk of Cow 10 on August 18, p.m., gave an analytical figure of 7·32 for the percentage of fat. This abnormally high figure appeared to be due to an error in analysis; but, using this percentage, the mean diameter was 3·41; whereas by the first method, not using the analytical figure, the mean diameter was 3·32. Such good agreement shows that the analytical figure was substantially correct.

[1] In our work published in 1911, p. 163, the centres of gravity of the curves A were given as representing the *mean* diameters. These are to be calculated from the number of globules in the area counted, and the percentage of fat. This will be rectified in due course. The use of the word "mean" was incorrect, and should have read "average." The distinction between these two terms, and their significance, is considered in detail in the account of the mensuration of the globules, p. 372.

[2] An important, and a very disconcerting source of error was experienced. The fat was determined in a centrifugal apparatus, using the amyl alcohol and sulphuric acid method. The milks, at first, showed great discrepancies. The tubes were then standardised, using a milk checked by the Adams method, and the following correction factors were found necessary:—1·03, 1·23, 1·18, 1·06, 1·10, 1·04.

It might be pointed out that the second method is, practically speaking, a modification of Babcock's original method, but the volume measured is much larger—about 10 times.

The results obtained from these feeding experiments have been tabulated, and, in order that they may be seen at a glance, curves have been drawn, as given below, pp. 350, 351.

Before discussing these tables, it should be pointed out that the number of cows was very small. But, although the calculations have been considerably simplified, owing to the adoption of the photographic method, yet the work is very laborious and all our available time was occupied. Moreover, the cost of the work was considerable, and would have been excessive had more cows been taken.

TABLE V.

Cow 7. Calved "lately" (? April).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
22. 7. 12	22·5	3·33	1677	2·94* (3·94)	13·0	4·22	1397	3·38* (3·76)
25. 7. 12	23·25	3·0	—	—	16·25	3·77	—	—
28. 7. 12	22·4	2·83	—	—	15·4	3·44	—	—
31. 7. 12	20·5	3·22	1296	3·16 (3·12)	14·3	4·22	1740	3·16
3. 8. 12	22·3	3·39	1710	2·93	15·2	3·51	1609	3·03
6. 8. 12	22·3	3·07	927	3·48	14·7	3·84	1140	3·50
9. 8. 12	22·7	—	—	—	15·5	—	—	—
12. 8. 12	23·5	2·76	1019	3·25	14·7	3·90	1417	3·27
Food modified mid-day Aug. 13								
15. 8. 12	23·4	2·67	1230	3·01	16·3	3·84	1335	3·32
18. 8. 12	24·25	2·94	1527	2·91 (2·93)	16·75	3·72	1709	3·03 (3·24)
Food changed Aug. 18								
30. 8. 12	22·1	3·12	1573	2·94	14·7	3·48	1627	3·01

Table VI.

Cow 8. Calved "some time ago" (? March).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
22. 7. 12	16·5	2·41	403	4·58* (5·48)	9·5	3·99	430	4·91* (5·58)
25. 7. 12	17·7	2·52	—	—	10·1	5·29	—	—
28. 7. 12	17·2	2·50	—	—	8·8	4·83	—	—
31. 7. 12	16·7	2·44	258	4·92 (5·11)	9·1	5·16	509	5·05
3. 8. 12	17·7	2·78	363	4·60	9·3	4·52	578	4·63
6. 8. 12	16·7	2·71	297	4·87	9·25	4·44	328	5·57 (5·72)
9. 8. 12	16·3	—	—	—	9·1	—	—	—
12. 8. 12	16·8	2·94	523	4·15	8·25	3·84	494	4·62
Food modified mid-day Aug. 13								
15. 8. 12	16·8	2·46	288	4·77	9·3	4·14	399	5·09
18. 8. 12	17·75	1·98	314	4·32* (4·91)	10·3	5·16	721	4·50 (4·20)
Food changed Aug. 18								
30. 8. 12	16·6	2·52	341	4·55	9·4	4·56	677	4·41

Table VII.

Cow 9. Calved "considerable time ago" (? Nov. or Dec.).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
22. 7. 12	12·0	2·61	1481	2·82 (3·08)	7·0	3·66	1729	3·00 (3·19)
25. 7. 12	13·5	2·89	—	—	6·5	3·92	—	—
28. 7. 12	12·25	2·83	—	—	6·0	4·77	—	—
31. 7. 12	11·0	3·11	1940	2·74 (2·98)	5·8	4·61	2133	3·02
3. 8. 12	13·1	2·94	1488	2·93	6·6	4·26	1727	3·15
6. 8. 12	12·3	3·00	1162	3·20	5·5	4·03	1353	3·36
9. 8. 12	11·7	—	—	—	6·2	—	—	—
12. 8. 12	10·8	3·36	2029	2·76	5·5	5·04	1384	3·59
Food modified mid-day Aug. 13								
15. 8. 12	10·5	2·94	1730	2·78	5·4	4·32	1790	3·13
18. 8. 12	11·9	2·82	1578	2·83 (2·90)	6·0	4·38	2273	2·91 (3·17)
Food changed Aug. 81								
30. 8. 12	11·7	4·08	1343	3·38	6·1	4·68	1461	3·44

TABLE VIII.

Cow 10. Calved "considerable time ago" (? March).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
22. 7. 12	12·5	4·02	1616	3·16* (1·49)	7·25	5·50	1943	3·30 (3·10)
25. 7. 12	13·9	3·66	1993	—	6·75	5·00	—	—
28. 7. 12	13·2	4·16	—	—	5·9	5·11	—	—
31. 7. 12	11·8	3·55	2061	2·38 (2·68)	5·9	4·44	2312	2·90
3. 8. 12	12·8	4·38	2504	2·81	5·8	5·21	2489	2·98
6. 8. 12	11·1	4·20	2086	2·95	5·9	4·56	2221	3·30
9. 8. 12	12·2	—	—	—	5·5	—	—	—
12. 8. 12	10·9	3·96	2220	2·83	5·3	5·64	1812	3·41
Food modified mid-day Aug. 13								
15. 8. 12	10·8	4·44	1612	3·27	5·1	6·24	1592	3·68
18. 8. 12	8·6	5·22	2411	3·02 (2·79)	4·3	7·32	2346	3·41 (3·32)
Food changed Aug. 18								
30. 8. 12	9·4	4·32	2538	2·79	4·9	5·52	2845	2·91

TABLE IX.

Cow 11. Calved "some time ago" (? Nov. or Dec.).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
22. 7. 12	8·75	3·32	1427	3·06 (3·23)	5·25	3·55	1538	3·09 (3·22)
25. 7. 12	10·9	3·00	—	—	5·6	3·66	—	—
28. 7. 12	10·5	3·93	—	—	5·2	3·89	—	—
31. 7. 12	9·75	3·61	1552	3·10* (3·61)	4·75	4·50	1665	(3·25)
3. 8. 12	10·9	3·11	1704	2·85	5·25	3·51	1852	2·89
6. 8. 12	10·75	2·88	1192	3·13	5·2	4·08	1799	2·44
9. 8. 12	10·1	—	—	—	5·4	—	—	—
12. 8. 12	10·6	2·94	1736	2·78	5·25	3·78	1516	3·16
Food modified mid-day Aug. 13								
15. 8. 12	10·75	2·88	1668	2·80	5·7	3·60	1644	3·03
18. 8. 12	11·3	2·40	1396	2·59 (2·53)	5·6	4·04	2261	2·83 (2·91)
Food changed Aug. 18								
30. 8. 12	9·6	3·69	1793	3·04	5·0	3·96	2019	2·92

TABLE X.

Cow 12. Calved "lately" (? April).

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
22. 7. 12	12·0	3·44	300	5·27 (5·24)	8·0	4·88	544	4·85* (5·88)
25. 7. 12	13·5	3·89	—	—	8·25	4·72	—	—
28. 7. 12	14·0	3·11	—	—	8·0	4·99	—	—
31. 7. 12	11·2	3·16	300	4·36* (5·41)	8·25	4·77	608	4·64
3. 8. 12	15·1	3·62	339	5·14	8·4	5·49	688	4·66
6. 8. 12	14·7	3·00	237	5·44	7·0	5·52	530	5·09
9. 8. 12	13·5	—	—	—	7·5	—	—	—
12. 8. 12	13·7	2·88	229	5·43	7·6	5·16	494	5·10
Food modified mid-day Aug. 13								
15. 8. 12	13·3	2·70	196	5·59	8·2	4·56	516	4·82
18. 8. 12	13·25	3·66	527	4·46 (4·92)	7·1	4·80	—	—
Food changed Aug. 18								
30. 8. 12	12·25	3·60	416	4·80	6·5	6·36	605	5·12

The tables include determinations made upon the morning and the evening milk; for the curves, however, an average of these two daily measurements was taken. The "average yield of milk" represents the daily average for the three-day period. The fat was estimated on the centrifuge. The number of globules was determined, as already mentioned. The mean diameters were worked out from the number of globules and the percentage of fat—method B. Those figures for the mean diameter given in brackets were calculated from the actual measurements of the globules—method A. On August 13 (mid-day) the food was slightly modified; the rations were changed over on August 18, as shown.

One of the most noticeable features of the tables is that the numbers for each cow are very irregular, whether considering the fat, the number of globules, or the diameter. This is probably due to the examination of too small a volume of milk.

With regard to the curves, which have been plotted from these figures, in order to reduce any individual peculiarity of the cows, an average given by the two cows of each set has been plotted in the upper of the three curves (*a*). This curve, of course, does not give any absolute value, but it does show the variations during the period of the experiments.

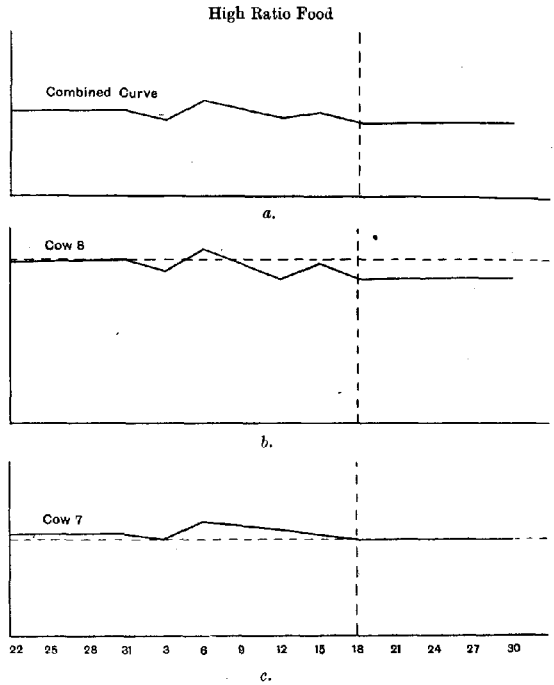

Fig. 3. Curves showing variation in mean size of fat globules during feeding experiments. The perpendicular dotted line indicates the point at which the High Ratio Food was changed to Low Ratio Food. Mean size of globules plotted as ordinates; dates as abscissae.

One would expect that the globules would diminish in size in any case, owing to the advancement of the period of lactation; but the curve is so irregular that one cannot observe a diminution in size, even due to this factor. Equally irregular variations have been experienced by others, as will be seen by an examination of Gutzeit's figures.

The only conclusion which can be drawn from these experiments is that the food has little or no influence on the size of the globules, if the "ratio" is taken as the criterion.

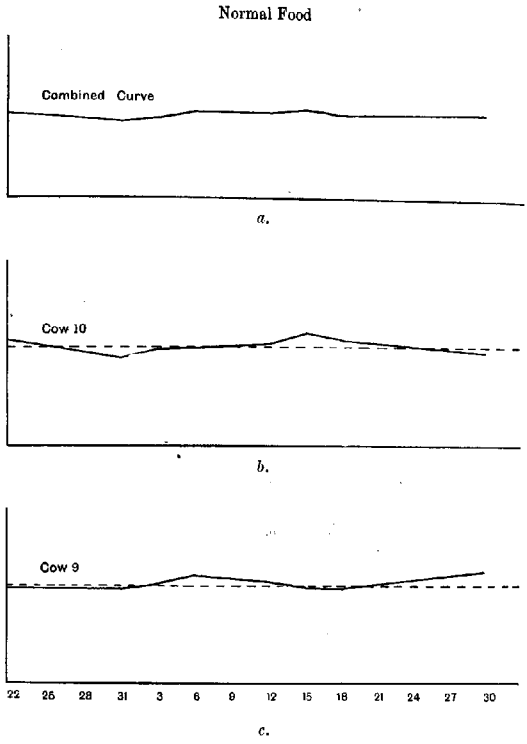

Fig. 4. Curves showing variation in mean size of fat globules during feeding experiments (control). Mean size of globules plotted as ordinates; dates as abscissae.

It is to be noted, however, that Woll (Digestion Expts., *Seventh Ann. Report, Agric. Expt. Stat., Wisconsin*, 1890, 238; also *Agricultural Science*, VI, 1892, 545) stated that it was *dry* food which gave the larger globules, but no information is given as to the *food ratio*. In our case, all the cows received grass, but the proportions were greater for those which had the food with low ratio; moreover, these cows had no cake, but maize meal and hay in place of it.

A plain food of grass only in the one case, and of dry foods only in the other, was not given in this series of experiments, because it was asserted by the herdsman that the cows would "go off their milk," owing to the character of their previous feed.

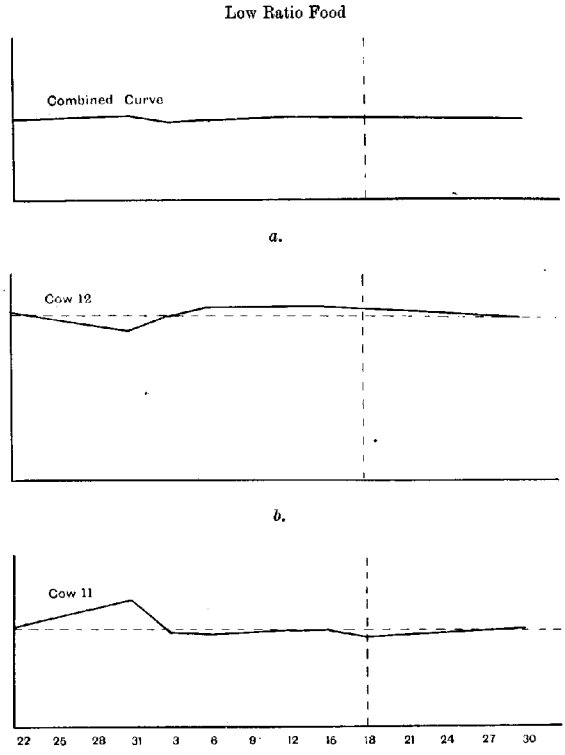

Fig. 5. Curves showing variation in mean size of fat globules during feeding experiments. The perpendicular dotted line indicates the point at which the Low Ratio Food was changed to High Ratio Food. Mean size of globules plotted as ordinates; dates as abscissae.

It is proposed to repeat the experiments, giving some cows a "dry" food and others grass only. In these experiments, it is suggested that observations should be made on the effect of the foods

upon the character of the serum and upon the churnability; for, in connection with previous experiments, it has been noted that there seems to be some relationship between the time of churning, the colour and quality of the butter, and the size of the globules. It is proposed also to make more determinations on each sample, taking them at longer intervals. Referring to the Tables V—X once more, it is to be noticed that there is a considerable difference in the mean diameter of the globules of the morning and evening milk on the same day; generally, those of the afternoon are the larger.

It may be observed, also, that in a number of the samples the percentage of fat is below the Government standard, and that in some it is abnormally high—a fact which can scarcely be due to sampling, as this was done within a short time of milking.

The number of globules in the milk of the same cow, at different periods, varies very considerably; in one case, a variation from 328 to 721 globules per unit volume in twelve days.

The results obtained from the cows at Dorking have been tabulated and curves drawn from them (Tables XI—XIII and Fig. 6). Here, again, it would appear that the feeding has had no effect on the globules. In this case, one may fairly presume that the cows had a very much larger quantity of *wet* food, yet no diminution in size of the globules is to be observed. With these cows, there is a much greater regularity in the size of the globules.

TABLE XI.

Cow 1. Jersey.

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
Aug. 3	18·3	3·30	2167	2·68	12·0	4·98	3130	2·72
,, 6	17·7	—	—	—	12·4	4·68	3467	2·58
,, 9	17·75	—	—	—	11·5	4·62	—	—
,, 12	18·0	3·72	2633	2·62	11·5	4·74	3085	2·69
,, 15	17·1	3·96	1859	3·00	10·5	5·10	2672	2·89
,, 18	16·8	3·96	2397	2·76	11·1	4·92	2793	2·82

TABLE XII.

Cow 2. Guernsey.

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
Aug. 3	15·9	3·90	1526	3·19	9·75	5·04	1540	3·46
,, 6	15·9	4·38	1853	3·11	10·1	4·14	2117	2·92
,, 9	16·1	—	—	—	9·9	5·22	—	—
,, 12	16·0	3·96	1632	3·14	9·7	4·50	1685	3·24
,, 15	15·75	—	—	—	8·9	4·74	1420	3·49
,, 18	15·4	4·32	1487	3·28	9·5	4·92	1841	3·24

TABLE XIII.

Cow 3. Guernsey.

Date	Morning				Afternoon			
	Average daily yield of milk	Fat	No. of globules	Mean diameter	Average daily yield of milk	Fat	No. of globules	Mean diameter
	lbs.	%		μ	lbs.	%		μ
Aug. 3	16·75	4·80	1099	3·81	10·9	5·76	1347	3·79
,, 6	16·8	3·96	1085	3·59	11·25	—	—	—
,, 9	16·6	4·62	—	—	9·9	5·10	—	—
,, 12	15·0	5·40	1415	3·65	10·5	7·08	1557	3·87
,, 15	15·2	4·68	1237	3·64	9·5	6·00	1345	3·84
,, 18	14·8	4·80	862	4·14	9·7	6·06	1097	4·13

There is one point which appears to be of considerable economic interest. It is generally considered that Jerseys and Guernseys give milks with large globules, which cream well, whereas Shorthorns occupy a medium position. In the work of this year, the mean sizes of the globules are remarkable; the Jerseys and Guernseys are in agreement with the usual values, whereas those of the impure

Shorthorn are not only considerably larger than those of the Jerseys, but also they are larger than any mean sizes which have been seen by us, or recorded by Gutzeit, for any breed. Woll (*Eleventh Ann. Report, Agric. Expt. Stat., Wisconsin*, 1894, 230), however, records a Shorthorn milk having a mean size globule of 7·5μ, and gives other measurements of the same order as ours.

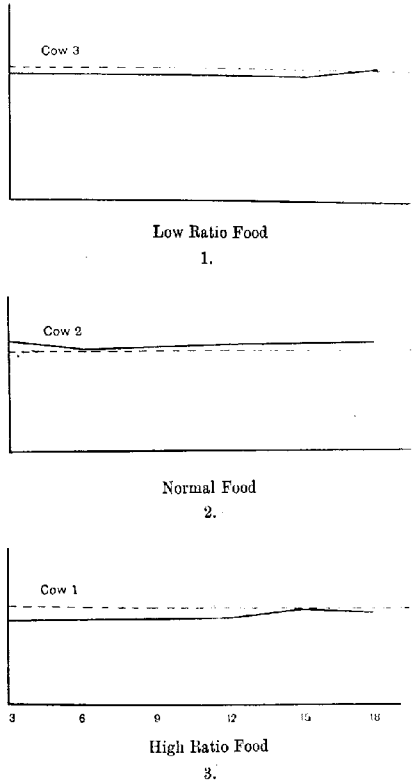

Fig. 6. Curves showing variation in mean size of fat globules during feeding experiments at Dorking. Mean size of globules plotted as ordinates; dates as abscissae.

These high values can scarcely be due to errors in counting, as they occur frequently. Neither can it be due to sampling, which might be the case if the milk had been standing some time before sampling,

when the upper layer would contain a greater number of larger globules, and also if the sample had been taken from this upper layer. The fact that an abnormally high percentage of fat was not found, precludes this possibility; on the contrary, the largest mean size occurs in the morning milk of cow 12, when the percentage of fat is the lowest recorded for that milk (2·70%).

Not only is it our experience that Shorthorn milks sometimes have a mean globule considerably larger than those of an average Jersey milk, but Woll (*Eleventh Ann. Report, Agric. Expt. Stat., Wisconsin*, 1894, 230), making determinations upon milks from a whole herd (99 cows), obtained similar figures.

If the size of globule is an important factor in dairying—as many consider—the question arises as to whether the *strain* of the cow may not be of more importance than the actual *breed*. That is to say, though, generally speaking, Jerseys are superior to Shorthorns for dairy work, yet certain *strains* of Jerseys may be inferior to certain *strains* of Shorthorns, and so with other breeds.

The only other suggestion bearing on this point, which we can find, is that contained in a paper of Cederholm (*U.S.D.A. Expt. Stat. Record*, XII, 1901, 482). He estimated the fat in the several cows' milks, and then that in the milks of their daughters by different bulls. His results show that each bull almost always causes an increase or a decrease in the percentage of fat. He concludes therefore "that the bull exerts a decided influence for better or worse on the milk product of his progeny."

This raises the question—important in its practical aspect—as to whether more attention should not be given to this point in attempting to improve our dairy herds. It is considered in actual practice, but scarcely sufficiently in interpreting experimental results.

THE ENUMERATION AND MEASUREMENT OF FAT GLOBULES IN MILK.

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In investigating the influence of various factors on the character of milk, it is now recognised that it is insufficient (at least for many purposes) to make comparisons of the percentage of fat, but some regard must be paid to the distribution of the fat in the globules. The technique of such investigations is somewhat complicated, and, as it was impossible to include in our previous papers a full description of the many points involved, it has been thought desirable to give the working details of the method employed, and at the same time to compare it with the method employed by previous workers.

It is hoped that these details may prove of considerable assistance to subsequent workers on similar problems.

Broadly speaking, there are two general methods for the enumeration and measurement of fat globules in milk; in one, the milk is examined in a capillary tube; in the other, in a flat cell. Both these methods were originally devised for the counting of corpuscles in blood, and have been adopted, with slight modifications, for milk examinations.

The capillary tube method was introduced for milk examinations by Bouchut, but, as Babcock made many extensive investigations with it, the method is usually associated with his name.

Babcock's original paper (*Fourth Annual Report, N.Y. Expt. Station, Geneva*, 1886, pp. 226, 275) is not generally available. Gutzeit, who has employed Babcock's method in a very exhaustive investigation on milk globules, has published, with his results, a full description of the method (*Landwirtschaftliche Jahrbücher*, 1895, 545). Since this journal is not easily accessible in this country, the authors

have made a detailed abstract of Gutzeit's description of Babcock's method.

The present authors have employed the flat cell method, with certain modifications, as it appears to present distinct advantages over that of Babcock.

I. Babcock's Method of Investigation.

Detailed Abstract from Gutzeit.

In order to measure the mean diameter of fat globules by Babcock's method, the number of globules in a measured volume of milk is determined. For this purpose, glass capillaries, of about 0·1 mm. diameter and 2—3 cms. long, are employed, the diameter being as constant as possible over small lengths.

10 ccs. of milk are diluted in a graduated flask to 500 ccs. With rich milk, or towards the end of the period of lactation, 5 ccs. may be used.

The capillary tubes are filled by dipping the end into the well mixed diluted milk, and the ends of the tubes are then sealed by melting in a small flame. The tubes are placed side by side, on a microscope slide, and fixed at both ends with a drop of melted wax. The slide is allowed to remain for half an hour on the microscope stage, which must be perfectly horizontal; a drop of water (or glycerine) is placed on the tubes, which are then covered with the cover-slip. By using an objective of sufficient magnification, the fat globules can be clearly recognised and counted through the capillary wall.

If the inner transverse diameter of the capillary is read off in terms of the divisions of the ocular micrometer, the ocular scale being at right angles to the length of the tube, and the micrometer focussed on the two bounding lines, the volume in which the enumerated globules are suspended can easily be calculated.

As we shall see later, the two lines do not represent the actual limits of the capillary walls, though they bear a definite relationship to them; their relative position is not affected, if the convergency of the illuminating rays (aperture of the sub-stage condenser) is altered, as would be the case if it were an interference phenomenon. Figure 1 shows the appearance of such a tube under the microscope at a magnification of 400 diameters, with an image of the ocular micrometer superposed.

A tube, 100 micrometer divisions long and 100 divisions broad, is taken as a *unit* tube; and the number of globules which would occur in a volume of milk required to fill such a tube is calculated by multiplying by 100^2 the number of fat globules found in a capillary 100 divisions long, and dividing by the square of the diameter of the capillary, measured in micrometer divisions.

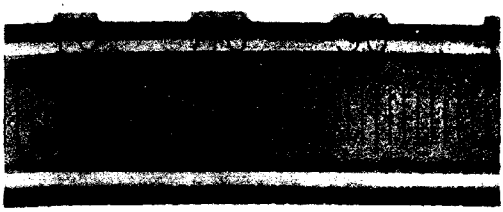


Fig. 1.

Thus, if a represents the number of enumerated globules and d the internal diameter of the tube, the number of globules occurring in a *unit* tube is given by the formula $\frac{a \times 10{,}000}{d^2}$, and, provided investigations are always carried out at the same dilution and magnification, the various values given by this formula may be directly compared.

In Gutzeit's experiments, one micrometer division corresponded to 0·0025 mm.; in Babcock's and Woll's work, to 0·0024 mm.

If d represents the internal diameter of the tube—measured in micrometer divisions—and the length of the tube always corresponds to 100 divisions, then the volume (J) of the tube used for each measurement is given by the equation

$$J = \frac{d^2 \times \pi \times 100 \times (0{\cdot}0025)^3}{4} \text{ mm.}^3$$

If, in this volume, a globules are counted, then 0·0001 mm.^{3} of the diluted milk will contain

$$\frac{a \times 4 \times 0{\cdot}0001}{d^2 \times \pi \times 100 \times (0{\cdot}0025)^3} \text{ fat globules,}$$

and, since the milk has been diluted 50 times, 0·0001 mm.³ of milk will contain

$$\frac{a \times 4 \times 0{\cdot}0001 \times 50}{d^2 \times \pi \times 100 \times (0{\cdot}0025)^2} = \frac{a \times 4074}{d^2}$$

fat globules. 0·0001 mm.³ is taken as the "standard quantity," and the number of fat globules occurring in this quantity is designated by N. Thus

$$N = \frac{a \times 4074}{d^2}.$$

In order to decrease the error due to the uneven distribution of the fat globules, 3 counts on each tube were made, thus 9 counts in all; 15 in some very careful estimations (Babcock and Woll made 5 counts on each of 3 tubes, but their micrometer scale was only half as long as Gutzeit's).

An example is given below.

Montavon Cow "Rezia." *20th August.*
Fat content 3·22 %. *Milk diluted* 50 *times.*

Capillary No.	I	II	III
d =	47	49	47
1st reading a =	124	150	177
2nd reading a =	190	170	153
3rd reading a =	183	194	166
Total	497	514	496
Mean	166	171	165

Using the formula previously given, $\frac{a \times 10{,}000}{d^2}$, these readings give 737 as the mean figure; that is to say, there are 737 fat globules contained in a *unit* tube. This number multiplied by the factor 0·4074, obtained above, gives 300 as the number of fat globules (N) occurring in 0·0001 mm.³ of milk. Further, if the fat content of the milk ($= f$) is known, there are the following relationships.

In 0·0001 mm.³ of milk there are $\frac{0{\cdot}0001 \times f}{100}$ mm.³ of fat. This quantity of fat corresponds to the quantity in N globules. The volume

of the mean size globule is, therefore, $\frac{0{\cdot}0001f}{100N} = 0{\cdot}000{,}001 \times \frac{f}{N}$ mm.^{3} This expression represents Babcock's "relative size." In the example given $f = 3{\cdot}22\ ^0/_0$, $N = 300$, the relative size

$$= \frac{3{\cdot}22}{300} \times 0{\cdot}000{,}001 \text{ mm.}^3 = 0{\cdot}000{,}000{,}0107 \text{ mm.}^3$$

As Babcock himself pointed out, this expression does not give absolute results, since the fat content is expressed in weight percentage, and no allowance for the specific gravity has been made. If the mean specific gravity of milk is taken as 1·0315, and that of butter fat as 0·94 at 15°, the volume of the mean size globule (Vd) is obtained by multiplying the value for the relative size by $\frac{1{\cdot}0315}{0{\cdot}94} = 1{\cdot}1$. This figure is used as a constant throughout this work.

In the above example $Vd = 0{\cdot}000{,}000{,}0107 \times 1{\cdot}1 = 0{\cdot}000{,}000{,}0118 \text{mm.}^3$ From the mean volume, the mean diameter of the globules can be calculated, but as all variations in the mean size are more evident when expressed as volumes, Gutzeit, like Babcock and Woll, compared the mean volumes, and only exceptionally calculated the mean diameters.

In order to be able to deal better with the small numbers involved, the micromillimetre ($= \mu = 0{\cdot}001$ mm.) was taken as unit of length, and μ^3 as unit of volume. Vd in the above example $= 11{\cdot}8\mu^3$, the "standard quantity" for the count of globules $= 100{,}000\mu^3$, the division of the micrometer scale $= 2{\cdot}5\mu$, etc.

To obtain a clear image of the tubes, it is necessary to mount them in a liquid, as described; for this purpose Babcock and Woll used concentrated glycerine. The value for d varies according to the liquid used; the same tube for instance having an apparent diameter of 65 micrometer divisions in air, 56 in water, and 52 in glycerine. This phenomenon is due to the refraction of the light from the glass into the less dense liquid. Allowance can be made for it by dividing the diameter of the tube, read off on the micrometer, by the refractive index of the glass, of which the tube is composed, compared with that of the mounting liquid. If the mounting liquid, for instance, Canada Balsam, has the same refractive index as the glass, the measurements given by the microscope represent the real diameter of the tube. As a matter of fact, Gutzeit found it more convenient to mount the capillary tube in water, the index of refraction always being 1·333, and to calculate the value for d. For this purpose, the index of refraction

of the glass used was taken as identical with that of Canada Balsam, which was found, by means of an Abbe refractometer, to be 1·514; the refractive index of glycerine is 1·451. Therefore the factor required to convert d to an absolute value is $\frac{1{\cdot}333}{1{\cdot}514} = 0{\cdot}88$ if water is used, and $\frac{1{\cdot}451}{1{\cdot}514} = 0{\cdot}96$ if glycerine is used. These factors may be obtained without the use of a refractometer, by measuring the same tube in Canada Balsam and in water.

d in Canada Balsam = 50·3 $\quad \frac{50{\cdot}3}{56{\cdot}0} = {\cdot}89.$
d in water = 56·0

If absolute values are required, the diameter of the capillary having been determined in water or glycerine, N (the number of fat globules) must be divided by the square of the corresponding factor (0·88 in the case of water and 0·96 in the case of glycerine), and Vd (the mean volume) multiplied by it.

For instance, in the example previously given, a having been determined in *water* (as is the case throughout this work), the absolute value of

$$Vd = 11{\cdot}8 \times 0{\cdot}89^2 = 9{\cdot}51\mu^3.$$

It is obvious from the foregoing that, theoretically, Babcock's method, with the slight modifications introduced, is reliable, and should give absolute values. Woll's parallel estimations with it agree well, differing by only a few per cent. (*Agric. Science*, 1892, 445).

Considering the examples given above, it will be noticed that the readings in the same tube differ by about 30 °/₀; this, however, is not surprising, as, at a dilution of 50 or 100 times, a constant and equal distribution of the fat globules is not possible. The three figures which are obtained from the formula $\frac{N \times 10{,}000}{d^2}$, viz. 752, 712, 747, agree within 6 °/₀; in the worst cases this difference amounted to 10 °/₀. If we take two complete estimations with three capillaries and five readings on each, we find, for instance:

4th January, 1893. *Milk from Municipal Stall.* $f = 4{\cdot}51$ °/₀.

1st experiment $N = 488$, $Vd = 10{\cdot}19\mu^3$.
2nd experiment $N = 501$, $Vd = 9{\cdot}90\mu^3$.
Difference 13 $0{\cdot}3\mu^3$.

The difference between the two values for N is 13 globules; that between the two values for Vd, $0{\cdot}3\mu^3$, or only about 3 °/₀. Repeated

estimations gave similar results. Better results were usually obtained with a smaller fat content and a higher value for Vd.

More accurate values for Vd may be obtained, if desired, by employing a greater magnification and a more accurate measurement of d and by an increased number of counts on more than three tubes. As, however, the estimation of the fat, by means of the centrifuge, is only accurate to about 3% (calculated on the fat) even when the greatest care is taken, there is not much point in obtaining a more accurate figure for d. An idea of the accuracy of the value for Vd, as given by this method, may be obtained by calculating the mean diameter d from the formula $V = \frac{4}{3}\pi r^3$.

Estimation 1.	$d = 2{\cdot}69\mu$.
„ 2.	$d = 2{\cdot}66\mu$.
Difference	$0{\cdot}03\mu$.

Since it is impossible to measure beyond $0{\cdot}1\mu$, this difference is of no account.

In this connection, it may be mentioned that Vd for the same cow, within one lactation period, may vary as much as 300% from day to day.

Theoretically, a slight error might be introduced in the factor 1·1, as only mean values for the specific gravity of milk and for butter fat have been taken. The specific gravity of the milk could be determined easily in each case; that of the butter fat at 15° C. not readily. If, however, experiments are carried out over the whole period of lactation, such variations would be eliminated in the mean Vd.

The computation of the two values N and Vd is simpler than it appears. If, instead of reducing the value of d, measured in water, to an absolute figure, we divide the final factor by $0{\cdot}89^2$, we have

$$N = \frac{a}{0{\cdot}89^2 \times d^2} \times 0{\cdot}4074 = \frac{a \times 0{\cdot}5053}{d^2}.$$

Further, when the same number of readings are made with each of the three tubes, the divisions are saved, if the final factor is divided by 3×3 (= 9) for three readings, 3×5 (= 15) for five readings, and so on.

The following table has been constructed to indicate the constant, dependent upon the dilution of the milk, the number of tubes and the number of readings on each tube required in the foregoing equation.

No. of readings for each tube	Dilution	No. of tubes	F	$\log F$
3	1 : 50	2	·8422	·9254
	1 : 100	2	·1684	·2263
	1 : 50	3	·5614	·7493
	1 : 100	3	·1123	·0504
5	1 : 50	2	·5053	·7035
	1 : 100	2	·1010	·0048
	1 : 50	3	·3374	·3374
	1 : 100	3	·6748	·8291

In addition, to obviate the three divisions by d^2, the following table was used, in which, for each value of d, measured in micrometer divisions, the tubes being mounted in water, the value for $10 - 2 \log d$ can be read off.

d	$10-2\log d$	d	$10-2\log d$	d	$10-2\log d$	d	$10-2\log d$	d	$10-2\log d$
30	0458	40	7959	50	6020	60	4437	70	3098
31	0172	41	7744	51	5849	61	4293	71	2974
32	9897	42	7535	52	5670	62	4157	72	2853
33	9630	43	7332	53	5515	63	4013	73	2743
34	9371	44	7132	54	5352	64	3876	74	2615
35	9112	45	6936	55	5192	65	3742	75	2499
36	8873	46	6746	56	5036	66	3609	76	2384
37	8635	47	6558	57	4884	67	3478	77	2271
38	8404	48	6375	58	4731	68	3349	78	2158
39	8179	49	6196	59	4583	69	3223	79	2048

Translator's Note. The values given in this table are not those of $10 - 2 \log d$, but merely the complements of the mantissae of $2 \log d$. The object of this procedure is to confine the use of logarithms to additions, eliminating all subtractions.

A calculation, using the example previously given, follows:—

"Rezia." 20*th August.* $f = 3{\cdot}22$. *Milk diluted* 50 *times.*
3 *counts on* 3 *tubes.*

Capillary No.	I	II	III
$d =$	47	49	47
1st reading $a =$	124	150	177
2nd reading $a =$	190	170	153
3rd reading $a =$	183	194	166
Total	497	514	496

The corresponding value for $10 - 2 \log d$ is added to the logarithm of each of these three totals:

log 497	6964	log 514	7110
$10 - 2 \log d$ (47)	6558	$10 - 2 \log d$ (49)	6196
log 225	3522	log 214	3306

log 496	6868
$10 - 2 \log d$ (47)	6558
log 225	3516

The sum of these three figures is taken, $225 + 214 + 225 = 664$. To calculate N from this value, it is only necessary to multiply by the factor given in the table of constants above. Thus, for three readings on three tubes at a dilution of 1 : 50, the factor is 0·5614, the logarithm of which is 7493. To this is added the logarithm of 664,

log 5614	7493
log 664	8222
log 373	5715 = log N.

For the calculation of Vd, the logarithm of $f(=3{\cdot}22\%)$ is added to that of 1·1 and the complement of the mantissa of log N is also added. Thus:

log f	5079
log 1·1	0414
Complement of the mantissa of log N (5715)	4285
log 9·50	9778 = log Vd.

In addition to the determination of N and Vd, the percentage of the globules greater than 2·5μ and 5μ in diameter were calculated. For this purpose, a special count of the number of globules, exceeding one, and those exceeding two, divisions of the scale was made after each ordinary count.

Observations on the milk of "Rezia," 20th August, will again serve as an example:

	Tube No. I, $d=47$			Tube No. II, $d=49$			Tube No. III, $d=47$		
	a	$>2{\cdot}5\mu$	$>5\mu$	a	$>2{\cdot}5\mu$	$>5\mu$	a	$>2{\cdot}5\mu$	$>5\mu$
1st reading.....	124	55	4	150	43	6	177	48	4
2nd reading ...	190	65	5	170	60	4	153	55	5
3rd reading ...	183	63	4	194	87	6	166	74	8
Total	497	183	13	514	190	16	496	177	17

Total No.	$>2{\cdot}5\mu$	$>5\mu$	
497	183	13	$\frac{550\times100}{1507}=36{\cdot}5\,\%>2{\cdot}5\mu.$
514	190	16	
496	177	17	$\frac{46\times100}{1507}=3{\cdot}0\,\%>5\mu.$
1507	550	46	

As in counting the globules greater than two divisions we have also included those greater than one, the latter figure must be subtracted from the first to give the percentage required. Thus there are

$33{\cdot}5\,\%$ greater than $2{\cdot}5\mu$,
$3{\cdot}0\,\%$ „ 5μ.

After a little practice, the size of the individual globules can be estimated with a fair degree of accuracy, as shown by the following example.

The figures, employed to demonstrate the accuracy of Vd, are again used.

1st experiment $N=488$; $Vd=10{\cdot}19\mu^3$; $31{\cdot}31\,\%>2{\cdot}5\mu$, $1{\cdot}90\,\%>5\mu$.
2nd experiment $N=501$; $Vd=9{\cdot}9\mu^3$; $31{\cdot}30\,\%>2{\cdot}5\mu$, $1{\cdot}95\,\%>5\mu$.

Further differentiation between the size of globules is not possible by this method, as, with such great dilutions, there are insufficient globules within the field of the micrometer. If, however, such an estimation appears desirable, the whole of the tube, covered with the slip, may be enumerated.

II. Flat Cell Method.

In outline, the method consists in making a photomicrograph of the milk, contained in a Thoma-Zeiss Cell, the enumeration and measurement being carried out on a print.

A. *Photomicrography of the Milk.*

The apparatus and method employed are as follows:—

Apparatus.

(i) A large *Zeiss Photomicrographic apparatus* is used. This consists of two benches, one supporting the entire optical system—the other the camera. For a full description and illustration of this apparatus, the reader is referred to Zeiss' Catalogue.

The microscope used was a No. 4 projection ocular. The extension of the camera was such as to give a magnification of 500 diameters.

The cell, containing the milk, must be kept in a horizontal position, in order to prevent the fat globules collecting together in one portion

of it. The microscope is therefore used in a vertical position and a reversing prism is employed to render the beam horizontal.

(ii) *Illumination.* This is obtained from a hand-fed arc (carbons at right angles), burning about 13 ampères.

(iii) *Exposure.* As the fat globules are in continual movement (Brownian), a short exposure is necessary. A Unicum Shutter is interposed in the beam of light, and adjusted to give an exposure of about 1/50 second. The plates used were Paget Extra Special Rapid (Speed about 450 H. and D.), and a normal metol-hydroquinone developer was employed.

(iv) *The Cell.* The Cell is a specially shallow Thoma blood counting cell, the depth being 0·015 mm. (15μ). The area of each square is 1/400 sq. mm.; so that the side of one square is 0·05 mm. An area of 16 such squares is photographed, so that, at a magnification of 500 diameters, the side of the large square used for counting is

$$0{\cdot}05 \times 4 \times 500 = 100 \text{ mm. (or about 4 inches).}$$

Such a square is conveniently photographed on a half plate.

Method.

(i) *Adjustment of Magnification.* The clean empty cell is placed on the microscope stage and adjusted so that an image of the ruling of the squares is projected on to the ground glass screen of the camera which is then extended to give a magnification of exactly 500 diameters.

(ii) *Preparation of the Cell.* The glass slide and cover slip are both washed with soap and water (on a piece of cotton wool), thoroughly rinsed with water, and dried with a soft cloth.

The sample of milk is shaken sufficiently to ensure thorough mixing, but care must be taken not to churn it. A small portion is transferred, by means of a platinum loop, on to the centre of the cell, the cover slip is then lowered (by means of a needle) on to the slide and pressed gently, near the edges, till perfect contact is obtained. This is the case when Newton's rings can be seen at the surface of contact of the slide and cover slip. Some practice is necessary to judge the correct quantity of milk to be taken for the cell preparation, but, when the loop has once been adjusted, this is a very simple matter.

(iii) *Focussing.* In focussing the image on the ground glass screen, some difficulty is encountered, as, owing to the refraction of the light by the globules, no very definite outline can be obtained. Moreover, the globules float up to the underside of the cover-glass, so that the small globules are not in exactly the same plane as the large ones. With practice, however, very good results with sharp outlines can be obtained.

B. *Enumeration of the Fat Globules.*

The distribution of the fat in the various sized globules can be determined from the photographs in two ways:

(i) The *total number* of globules is determined in a definite area of the photograph—and therefore in a known volume of the milk. This figure, and the percentage of the fat as given by analysis, will give the "mean-diameter" of the globule.

(ii) The *diameter of each globule* in a definite area of the photograph is measured; and from this, without the aid of any analytical figure, the actual distribution of fat in the various sizes of globules can be computed.

The two methods are detailed below.

Method 1.

The photomicrographic print, obtained as already described, is ruled to correspond with 16 of the squares on the cell. Thus at 500 diameters magnification, the side of each small square will measure 25 mm., and the side of the square containing 16 of these will be 100 mm.

The total *number* of globules in each of the 16 small squares is then counted, each globule being marked off with a pencil as it is recorded; this ensures that each globule is counted once, and once only. The numbers for each of the 16 squares are then added, giving the total number for the whole area. Any globules cutting either of two adjacent sides of the large square (e.g. the top and left hand side) are included; whereas those cutting either of the two remaining sides are neglected.

The percentage of fat in the milk is determined by the centrifuge method.

Since all measurements made on the photograph relate to volumes, and since the analytical result is expressed as Wt/Wt percentage, a correction must be applied to correlate the two sets of figures.

Specific gravity of milk fat was taken to be 0·930.
" " milk was taken to be 1·034.

Then to convert Wt/Wt percentage to Vol/Vol percentage multiply by

$$\frac{1{\cdot}034}{{\cdot}930} = 1{\cdot}1118,$$

[log = 0·0460376].

Volume of the Cell.

16 squares are measured and the area of each square $= \frac{1}{400}$ mm.^{2}

The depth of the cell is 0·015 mm.

Therefore the volume measured is

$$\frac{16 \times 0{\cdot}015}{400} \text{ mm.}^3 = 0{\cdot}0006 \text{ mm.}^3 \ (= 600{,}000\mu^3).$$

Calculation of the "Mean Diameter."

If d and v represent respectively the diameter and volume of a sphere, then

$$v = \frac{\pi d^3}{6} \text{ or } d = \sqrt[3]{\frac{6v}{\pi}}.$$

Let $f = {}^{\circ}/_{\circ}$ of fat (Wt/Wt) found by analysis, and $n =$ No. of globules in the measured volume, then $100\mu^3$ of milk contain $f \times 1{\cdot}1118\mu^3$ of fat.

Therefore $600{,}000\mu^3$ (the volume measured) contains

$$f \times 1{\cdot}1118 \times 6000\mu^3 \text{ of fat.}$$

Therefore 1 globule will contain $\frac{1{\cdot}1118 \times 6000}{n}\mu^3$ of fat. This represents the volume of the mean sized globule.

Therefore, if $D =$ diameter of the mean globule, then

$$D = \sqrt[3]{\frac{6 \times f \times 1{\cdot}1118 \times 6000}{n\pi}}.$$

In practice, logarithms are used for the calculation of D. An actual example will render the method of working quite clear.

Example.

A sample of milk contained 3·96 ${}^{\circ}/_{\circ}$ of fat, and the counted volume of the milk contained 1632 globules.

$$D = \sqrt[3]{\frac{6 \times 1{\cdot}1118 \times 6000}{\pi} \times \frac{f}{n}} = \sqrt[3]{\frac{6 \times 1{\cdot}1118 \times 6000}{\pi} \times \frac{3{\cdot}96}{1632}}.$$

log 6	= 0·7781513	log $\frac{6 \times 1{\cdot}1118 \times 6000}{\pi}$	= 4·1051794
log 1·1118	= 0·0460267	add log 3·96 (f)	= 0·5976952
log 6000	= 3·7781513		4·7028746
	4·6023293	Subtract log 1632 (n) =	3·2127202
log π	= 0·4971499	Divide by 3	3)1·4901544
	4·1051794		0·4967181

= log of 3·1385.

Hence $D = 3{\cdot}14\mu$.

In calculating a series of such figures, the factor $\frac{6 \times 1{\cdot}1118 \times 6000}{\pi}$ remains constant and so, once obtained, the logarithm of this factor is simply added to that of f, the logarithm of n is subtracted and the result divided by 3 to obtain the cube root.

Method 2.

In this method, not only is a determination made of the number of globules in the ruled area of the photograph, but the diameter of each globule is measured.

For this purpose, a transparency is made of a series of circles, to correspond with 1, 2, 3, ... μ diameter. The magnification of the photographs was 500 diameters, so that the smallest circle was 0·5 mm. or 500μ diameter; the next 1·0 mm.; 1·5 mm., and so on; corresponding to 1, 2, 3$\mu \times 500$. This transparency is superposed on the photograph and the diameter of one globule is measured; this is noted and the globule is crossed through with a pencil; another globule is measured, the diameter noted, the globule crossed through; and so on. In this manner, each globule is measured once and once only, and a complete record is obtained of the globules in the counted area. One small square (2·5 × 2·5 cms.) is enumerated before proceeding to another, the final result being obtained by the addition of the figures from the 16 squares.

This method of enumeration claims the attention of two workers, one making and calling out the measurements, the other recording them. It is quicker to select all the globules of one size first. After some practice, it is not found necessary to gauge every globule, but only the first few of each size. Since it is impracticable to differentiate between globules of less diameter than 1μ, all such are recorded as 1μ globules. Moreover, no fraction of μ is taken into account, but the nearest whole figure is recorded.

From the figures thus obtained, three results may be calculated:

(*a*) The mean diameter of the globules.

(*b*) The actual distribution of the fat in the various sizes of globules.

(*c*) The percentage of fat in the milk.

(*a*) *Calculation of "Mean Diameter."*

The volume of fat contained in a globule of any definite diameter, multiplied by the number of such globules present, gives the volume of

fat contained by all the globules of that size. This operation is repeated for each size of globule in the milk. The sum of these figures gives the total volume of fat present in the measured volume of milk. If the volume, so obtained, be divided by the total number of globules, the volume of the mean sized globule results; from this, it is a simple matter to calculate the diameter of the mean sized globule. The following example shows the method of calculating the volume of the various sized globules ranging from 1μ to 14μ in diameter, and also of calculating the diameter of the mean globule.

Diam. of globules μ	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of globules	512	342	241	155	72	45	20	19	3	3	1	1	0	0
Total No.	1414													

d	v	$\log v$	n	$\log (v \times n)$	$v \times n$
1	$\frac{\pi}{6}\mu^3$	1·7189986	512	2·4282686	268
2	$\frac{8 \times \pi}{6}\mu^3$	0·6220886	342	3·1561147	1132
3	$\frac{27 \times \pi}{6}\mu^3$	1·1503624	241	3·5323794	3407
4	$\frac{64 \times \pi}{6}\mu^3$	1·5251786	155	3·7155103	5194
5	$\frac{125 \times \pi}{6}\mu^3$	1·8159086	72	3·6732411	4712
6	$\frac{216 \times \pi}{6}\mu^3$	2·0534524	45	3·7066649	5089
7	$\frac{343 \times \pi}{6}\mu^3$	2·2542927	20	3·5553227	3592
8	$\frac{512 \times \pi}{6}\mu^3$	2·4282686	19	3·7070222	5094
9	$\frac{729 \times \pi}{6}\mu^3$	2·5817261	3	3·0588474	1145
10	$\frac{1000 \times \pi}{6}\mu^3$	2·7189986	3	3·1961199	1571
11	$\frac{1331 \times \pi}{6}\mu^3$	2·8431767	1	2·8431767	697
12	$\frac{1728 \times \pi}{6}\mu^3$	2·9565423	1	2·9565423	905
13	$\frac{2197 \times \pi}{6}\mu^3$	3·060827	0	0	0
14	$\frac{2744 \times \pi}{6}\mu^3$	3·1573827	0	0	0

The total number is 1414.

The volume of fat in the volume of milk measured is $33{,}106\mu^3$.

The volume of the mean globule is $\frac{33{,}106}{1414} = 23{\cdot}41\mu^3$.

The diameter of the mean globule $(D) = \sqrt[3]{\frac{6V}{\pi}} = \sqrt[3]{\frac{6 \times 23{\cdot}41}{\pi}} = 3{\cdot}55\mu$.

The value for the mean diameter as given by these two methods should be in a fairly close agreement, and where such is not the case, a further examination of the milk can be made. It should be noted that, in Method 1, the percentage of fat determined analytically has been employed; in Method 2, no analytical figure is required, so that if the results, given by the two methods, are in agreement, there is little question of their accuracy.

(*b*) *Distribution of the Fat in the various sized Globules.*

Referring to the previous table, it will be seen that the figures under "$v \times n$" are a measure of the quantity of fat present in any particular size of globule.

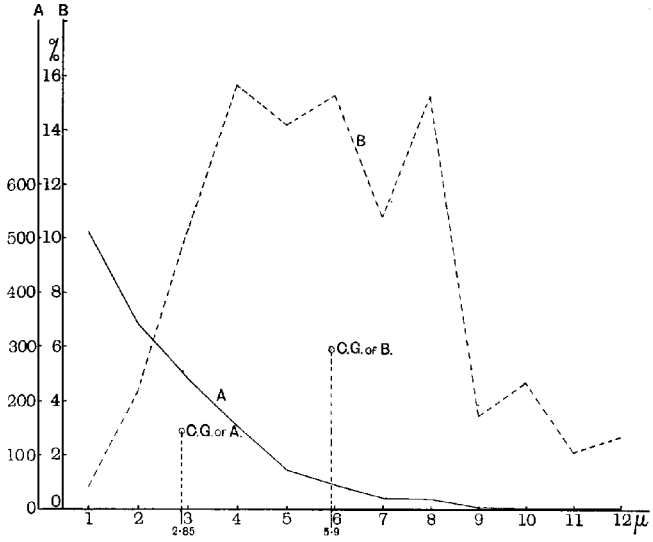
For instance, out of a total volume of fat of $33{,}106\mu^3$, $268\mu^3$ is present as 1μ diameter globules; $1432\mu^3$ as 2μ diameter globules; and so on. It is a simple matter to transform these numbers into percentages, as has been done in the following table:

Diameter μ	$v \times n$	% fat in various sized globules
1	268	0·81
2	1432	4·33
3	3407	10·29
4	5194	15·69
5	4712	14·23
6	5089	15·37
7	3592	10·85
8	5094	15·39
9	1145	3·46
10	1571	4·74
11	697	2·11
12	905	2·73
	33,106	100·00

From the last column, for instance, it will be seen that 15·37 % of the total fat is present in globules of 6μ diameter; 3·46 % in globules of 9μ diameter, and so on. A curve, connecting diameter and percentage of fat can be constructed from these figures. In figure 2, such a curve has been drawn (*B*), together with one showing the relationship between numbers of globules and diameters (*A*).

The centre of gravity of both curves is indicated. That of curve *B* represents the true "mean diameter"; that of curve *A*, the "average diameter," *i.e.* the value which would be arrived at from a purely visual examination, in which only two dimensions can be appreciated. The

large discrepancies between the values for these two centres of gravity is a forcible example of the futility of attempting to estimate the mean diameter by visual examination.



Diameter of globules

Fig. 2.

(c) The Percentage of Fat in the Milk.

The last column of the table shows that there are $33{,}106\mu^3$ of fat in the volume of milk measured. This volume has already been shown to be $600{,}000\mu^3$. Hence the percentage of fat (Vol/Vol) in the particular sample of milk is

$$\frac{33{,}106}{6000} = 5{\cdot}52\ \%\ \text{(Vol/Vol)}$$

$$= 4{\cdot}96\ \%\ \text{(Wt/Wt)}.$$

The actual figure furnished by analysis was

$$4{\cdot}80\ \%\ \text{fat (Wt/Wt)}.$$

These two figures should be identical, but if the small volume of milk measured (only 0·0006 mm.^{3}), and the difficulty of accurate measurement of the globules are considered, it will be seen that the agreement is as close as could be expected.

If the figure obtained by the above method differs considerably from that obtained by analysis, there is obviously an error in the determination; such an error may occur

(i) in the determination of fat,
(ii) in the selection of the sample for photography,
(iii) in the measurement of the globules.

Degree of accuracy of counting method.

It was considered desirable to test the reliability of the method, and, to this end, three separate cell preparations and photographs were made on one sample of milk, and three more on another sample. The globules in each of the three photographs were counted, and the resulting figures were compared.

Number of globules in counted area of photograph.

Count	Sample *A*	Sample *B*
1	2105	302
2	2286	345
3	2288	397
Mean	2229	348

The difference between the numbers obtained from the triplicate photographs may be explained by

(i) Errors in sampling.
(ii) Slight difference in focussing the image.
(iii) Errors in counting.
(iv) Lack of "clearness" and density in the print.
(v) Errors due to variation in the volume measured, owing to variation in temperature.

With care, the last three of these possible sources of error can practically be eliminated; the first is probably the most important, as only 0·0006 mm.^{3} of milk is photographed.

III. Conclusions.

Regarding the relative merits of the two methods, the authors have worked with both, and their preference for the cell method is based upon the following considerations.

In Babcock's method, measurements are made upon varying volumes of diluted milk, necessitating the use of a variable factor in the calculation of the mean volume.

In the cell method, a constant volume of undiluted milk is employed, and hence a constant factor is applied in all calculations. Moreover, the latter method dispenses with the use of an immersing liquid, and accordingly no correction for refraction is necessary.

Again, the volume of milk actually examined in the cell method is roughly ten times that measured in the capillary method.

Whilst several workers have made use of the cell method for milk globule enumerations, the application of photography to it is, as far as the authors are aware, novel. The innovation has several advantages. Foremost amongst these is the fact that permanent records are obtained, which may be enumerated at leisure. Since the film of milk photographed is so thin, no time is wasted waiting for the globules to rise (as is the case in the capillary method), and it is possible to make a very large number of exposures in a short period, the development, printing, counting, etc., being postponed, if necessary.

In Babcock's method, the effect of Brownian movement upon the smaller globules is liable to introduce two sources of error in counting. Firstly, the number of globules in the counted portion of the capillary is always changing, owing to the small globules at the limits of the measured length constantly leaving and re-entering the enumerated volume. Secondly, this constant oscillatory movement renders the counting of the small globules a matter of uncertainty. Since the calculation of Gutzeit's mean volumes (*Vd*) rests upon the total number of globules counted, and the percentage of fat, it follows that the omission of a minute globule is as serious as that of a very large one.

Gutzeit himself points out that there is a difference of about 30 %, in his parallel estimations, so that the above criticism is justified.

The use of a sufficiently short exposure in the photographic method completely obviates this difficulty. The parallel counts given on p. 374 differ by about 14 % respectively—a much better agreement than Gutzeit obtained.

Another very distinct advantage afforded by the photographic method is, that it is possible, by making the double enumeration, as already described (p. 368), to check off the results, and so discard any doubtful values.

In spite of the fact that the method adopted by the authors involves the use of expensive apparatus, and the expenditure of a considerable amount of time, yet they are of the opinion that its advantages far outweigh these objections. It is true that Woll (*Agricultural Science*, 1892, p. 441) compared the capillary and cell methods and

concluded in favour of the former. He found that he always detected more globules, when using the capillary, than when using the cell. This is not surprising, as an examination of his technique reveals the fact that in the former case he was using a magnification of 950 diameters, whereas, in the latter, the magnification was only 400 diameters. Gutzeit appears to have accepted Woll's valuation of the reliability of the two methods without question.

A fair comparison of the two methods indicates the distinct superiority of the procedure adopted by the authors.

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